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(54) **Compositions and methods for inhibiting expression of the PCSK9 gene**

Zusammensetzungen und Verfahren zur Hemmung der PCSK9-Genexpression

Compositions et procédés d'inhibition de l'expression du gène PCSK9

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Description

Field of the Invention

[0001] This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of the PCSK9 gene and the use of the dsRNA to treat pathological processes which can be mediated by down regulating PCSK9, such as hyperlipidemia.

Background of the Invention

[0002] Proprotein convertase subtilisin kexin 9 (PCSK99) is a member of the subtilisin serine protease family. The other eight mammalian subtilisin proteases, PCSK1-PCSK8 (also called PC1/3, PC2, furin, PC4, PC5/6, PACE4, PC7, and S1P/SKI-1) are proprotein convertases that process a wide variety of proteins in the secretory pathway and play roles in diverse biological processes (Bergeron, F. (2000) J. Mol. Endocrinol. 24, 1-22, Gensberg, K., (1998) Semin. Cell Dev. Biol. 9, 11-17, Seidah, N. G. (1999) Brain Res. 848, 45-62, Taylor, N. A., (2003) FASEB J. 17, 1215-1227, and Zhou, A., (1999) J. Biol. Chem. 274, 20745-20748). PCSK9 has been proposed to play a role in cholesterol metabolism. PCSK9 mRNA expression is down-regulated by dietary cholesterol feeding in mice (Maxwell, K. N., (2003) J. Lipid Res. 44, 2109-2119), up-regulated by statins in HepG2 cells (Dubuc, G., (2004) Arterioscler. Thromb. Vasc. Biol. 24, 1454-1459), and up-regulated in sterol regulatory element binding protein (SREBP) transgenic mice (Horton, J. D., (2003) Proc. Natl. Acad. Sci. USA 100, 12027-12032), similar to the cholesterol biosynthetic enzymes and the low-density lipoprotein receptor (LDLR). Furthermore, PCSK9 missense mutations have been found to be associated with a form of autosomal dominant hypercholesterolemia (Hchola3) (Abifadel, M., et al. (2003) Nat. Genet. 34, 154-156, Timms, K. M., (2004) Hum. Genet. 114, 349-353, Leren, T. P. (2004) Clin. Genet. 65, 419-422). PCSK9 may also play a role in determining LDL cholesterol levels in the general population, because single-nucleotide polymorphisms (SNPs) have been associated with cholesterol levels in a Japanese population (Shioji, K., (2004) J. Hum. Genet. 49, 109-114).

[0003] Autosomal dominant hypercholesterolemias (ADHs) are monogenic diseases in which patients exhibit elevated total and LDL cholesterol levels, tendon xanthomas, and premature atherosclerosis (Rader, D. J., (2003) J. Clin. Invest. 111, 1795-1803). The pathogenesis of ADHs and a recessive form, autosomal recessive hypercholesterolemia (ARH) (Cohen, J. C., (2003) Curr. Opin. Lipidol. 14, 121-127), is due to defects in LDL uptake by the liver. ADH may be caused by LDLR mutations, which prevent LDL uptake, or by mutations in the protein on LDL, apolipoprotein B, which binds to the LDLR. ARH is caused by mutations in the ARH protein that are necessary for endocytosis of the LDLR-LDL complex via its interaction with clathrin. Therefore, if PCSK9 mutations are causative in Hchola3 families, it seems likely that PCSK9 plays a role in receptor-mediated LDL uptake.

[0004] Overexpression studies point to a role for PCSK9 in controlling LDLR levels and, hence, LDL uptake by the liver (Maxwell, K. N. (2004) Proc. Natl. Acad. Sci. USA 101, 7100-7105, Benjannet, S., et al. (2004) J. Biol. Chem. 279, 48865-48875, Park, S. W., (2004) J. Biol. Chem. 279, 50630-50638). Adenoviral-mediated overexpression of mouse or human PCSK9 for 3 or 4 days in mice results in elevated total and LDL cholesterol levels; this effect is not seen in LDLR knockout animals (Maxwell, K. N. (2004) Proc. Natl. Acad. Sci. USA 101, 7100-7105, Benjannet, S., et al. (2004) J. Biol. Chem. 279, 48865-48875, Park, S. W., (2004) J. Biol. Chem. 279, 50630-50638). In addition, PCSK9 overexpression results in a severe reduction in hepatic LDLR protein, without affecting LDLR mRNA levels, SREBP protein levels, or SREBP protein nuclear to cytoplasmic ratio. These results indicate that PCSK9, either directly or indirectly, reduces LDLR protein levels by a posttranscriptional mechanism

[0005] Loss of function mutations in PCSK9 have been designed in mouse models (Rashid et.al., (2005) PNAS, 102, 5374-5379., and identified in human individuals Cohen et al., (2005), Nature Genetics., 37, 161-165. In both cases loss of PCSK9 function lead to lowering of total and LDLc cholesterol. In a retrospective outcome study over 15 years, loss of one copy of PCSK9 was shown to shift LDLc lower and to lead to an increased risk-benefit protection from developing cardiovascular heart disease (Cohen et.al., 2006 N. Engl. J. Med., 354., 1264-1272.). Clearly the evidence to date indicates that lowering of PCSK9 levels will lower LDLc.

[0006] Recently, double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans.* dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., Curr. Biol. (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

[0007] Despite significant advances in the field of RNAi and advances in the treatment of pathological processes which can be mediated by down regulating PCSK9 gene expression, there remains a need for agents that can inhibit PCSK9

gene expression and that can treat diseases associated with PCSK9 gene expression such as hyperlipidemia.

Summary of the Invention

[0008] The invention is defined by the claims. The invention provides a solution to the problem of treating diseases that can be modulated by down regulating the proprotein convertase subtilisin kexin 9 (PCSK9) by using double-stranded ribonucleic acid (dsRNA) to silence PCSK9 expression.

[0009] The invention provides double-stranded ribonucleic acid (dsRNA) as defined in the claims, as well as compositions and in vitro methods for inhibiting the expression of the PCSK9 gene in a cell using such dsRNA. The invention also provides such as RNA for treating pathological conditions that can modulated by down regulating the expression of the PCSK9 gene, such as hyperlipidemia. The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an mRNA transcript of the PCSK9 gene.

[0010] In one embodiment, the invention provides a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human PCSK9 gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is fully complementary to at least a part of an mRNA encoding PCSK9, wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene by at least 40%, and wherein said part of an mRNA encoding PCSK9 consists of the sequence UCAUAGGCCUGGAGUUUAU.

[0011] The dsRNA molecules of the invention can be comprised of naturally occurring nucleotides or can be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative. Alternatively, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

[0012] In another embodiment, the invention provides an isolated cell comprising one of the dsRNAs of the invention. The cell is generally a mammalian cell, such as a human cell.

[0013] In another embodiment, the invention provides a pharmaceutical composition for inhibiting the expression of the PCSK9 gene in an organism, generally a human subject, comprising one or more of the dsRNA of the invention and a pharmaceutically acceptable carrier or delivery vehicle.

[0014] In another embodiment, the invention provides an in vitro method for inhibiting the expression of the PCSK9 gene in a cell, comprising the following steps:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA is as defined in the claims.

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the PCSK9 gene, thereby inhibiting expression of the PCSK9 gene in the cell.

[0015] In another embodiment, the invention provides the dsRNA defined in the claims for treating, preventing or managing pathological processes which can be mediated by down regulating PCSK9 gene expression, e.g. hyperlipidemia, comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention.

[0016] In another embodiment, the invention provides vectors for inhibiting the expression of the PCSK9 gene in a cell, comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

[0017] In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of the PCSK9 gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

Brief Description of the Figures

[0018]

Fig. 1 shows the structure of the ND-98 lipid.

Fig. 2 shows the results of the *in vivo* screen of 16 mouse specific (AL-DP-9327 through AL-DP-9342) PCSK9 siRNAs directed against different ORF regions of PCSK9 mRNA (having the first nucleotide corresponding to the

ORF position indicated on the graph) in C57/BL6 mice (5 animals/group). The ratio of PCSK9 mRNA to GAPDH mRNA in liver lysates was averaged over each treatment group and compared to a control group treated with PBS or a control group treated with an unrelated siRNA (blood coagulation factor VII).

Fig. 3 shows the results of the *in vivo* screen of 16 human/mouse/rat crossreactive (AL-DP-9311 through AL-DP-9326) PCSK9 siRNAs directed against different ORF regions of PCSK9 mRNA (having the first nucleotide corresponding to the ORF position indicated on the graph) in C57/BL6 mice (5 animals/group). The ratio of PCSK9 mRNA to GAPDH mRNA in liver lysates was averaged over each treatment group and compared to a control group treated with PBS or a control group treated with an unrelated siRNA (blood coagulation factor VII).
Silencing of PCSK9 mRNA resulted in lowering total serum cholesterol levels.
The most efficacious in terms of knocking down PSCK9 message siRNAs showed the most pronounced cholesterol lowering effect (around 20-30%).
Fig. 4 shows the results of the *in vivo* screen of 16 mouse specific (AL-DP-9327 through AL-DP-9342) PCSK9 siRNAs in C57/BL6 mice (5 animals/group). Total serum cholesterol levels were averaged over each treatment group and compared to a control group treated with PBS or a control group treated with an unrelated siRNA (blood coagulation factor VII).

Fig. 5 shows the results of the *in vivo* screen of 16 human/mouse/rat crossreactive (AL-DP-9311 through AL-DP-9326) PCSK9 siRNAs in C57/BL6 mice (5 animals/group). Total serum cholesterol levels were averaged over each treatment group and compared to a control group treated with PBS or a control group treated with an unrelated siRNA (blood coagulation factor VII).

Fig. 6 shows a comparison of the *in vitro* and *in vivo* results for silencing PCSK9.

Fig. 7A and Fig. 7B show *in vitro* results for silencing PCSK9 using monkey primary hepatocytes.

Fig. 8 shows *in vivo* activity of LNP-01 formulated siRNAs to pcsk-9.

Fig. 9 shows *in vivo* activity of LNP-01 Formulated chemically modified 9314 and 10792 parent molecules at different times. Clearly modified versions of 10792 display *in vivo* silencing activity.

Detailed Description of the Invention

[0019] The invention provides a solution to the problem of treating diseases that can be modulated by the down regulation of the PCSK9 gene, by using double-stranded ribonucleic acid (dsRNA) to silence the PCSK9 gene thus providing treatment for diseases such as hyperlipidemia.

[0020] The invention provides double-stranded ribonucleic acid (dsRNA) as defined in the claims, as well as compositions and in vitro methods for inhibiting the expression of the PCSK9 gene in a cell or mammal using the dsRNA. The invention also provides such as RNAs for treating pathological conditions and diseases that can be modulated by down regulating the expression of the PCSK9 gene. dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi).

[0021] The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an mRNA transcript of the PCSK9 gene. The use of these dsRNAs enables the targeted degradation of an mRNA that is involved in sodium transport. Using cell-based and animal assays, the present inventors have demonstrated that very low dosages of these dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the PCSK9 gene. Thus, the methods and compositions of the invention comprising these dsRNAs are useful for treating pathological processes which can be mediated by down regulating PCSK9, such as in the treatment of hyperlipidemia.

[0022] The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of the target PCSK9 gene, as well as compositions and methods for treating diseases that can be modulated by down regulating the expression of PCSK9, such as hyperlipidemia. The pharmaceutical compositions of the invention comprise a dsRNA having an antisense strand comprising a region of complementarity which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an RNA transcript of the PCSK9 gene, together with a pharmaceutically acceptable carrier.

[0023] Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, in vitro methods of using the compositions to inhibit expression of the PCSK9 gene, and as RNAs of the invention to treat diseases that can be modulated by down regulating

the expression of PCSK9.

I. Definitions

[0024] For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

[0025] "G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cyrtosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

[0026] As used herein, "PCSK9" refers to the proprotein convertase subtilisin kexin 9 gene or protein (also known as FH3, HCHOLA3, NARC-1, NARC1). mRNA sequences to PCSK9 are provided as human: NM_174936; mouse: NM_153565, and rat: NM_199253.

[0027] As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of the PCSK9 gene, including mRNA that is a product of RNA processing of a primary transcription product.

[0028] As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

[0029] As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0030] This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes of the invention.

[0031] "Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

[0032] The terms "complementary", "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

[0033] As used herein, a polynucleotide which is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide which is substantially complementary to a contiguous portion of the mRNA of interest (e.g., encoding PCSK9). For example, a polynucleotide is complementary to at least a part of a PCSK9 mRNA if the sequence is substantially complementary to a non-interrupted portion of a mRNA encoding PCSK9.

[0034] The term "double-stranded RNA" or "dsRNA", as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they

may be separate RNA molecules. Where separate RNA molecules, such dsRNA are often referred to in the literature as siRNA ("short interfering RNA"). Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop", "short hairpin RNA" or "siRNA". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs. In addition, as used in this specification, "dsRNA" may include chemical modifications to ribonucleotides, including substantial modifications at multiple nucleotides and including all types of modifications disclosed herein or known in the art. Any such modifications, as used in an siRNA type molecule, are encompassed by "dsRNA" for the purposes of this specification and claims.

[0035] As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule. For clarity, chemical caps or non-nucleotide chemical moieties conjugated to the 3' end or 5' end of an siRNA are not considered in determining whether an siRNA has an overhang or is blunt ended.

[0036] The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, e.g., within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

[0037] The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

[0038] "Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, dsRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection.

[0039] The terms "silence" and "inhibit the expression of" in as far as they refer to the PCSK9 gene, herein refer to the at least partial suppression of the expression of the PCSK9 gene, as manifested by a reduction of the amount of mRNA transcribed from the PCSK9 gene which may be isolated from a first cell or group of cells in which the PCSK9 gene is transcribed and which has or have been treated such that the expression of the PCSK9 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

[0040] Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to PCSK9 gene transcription, e.g. the amount of protein encoded by the PCSK9 gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g apoptosis. In principle, PCSK9 gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of the PCSK9 gene by a certain degree and therefore is encompassed by the instant invention, the assay provided in the Examples below shall serve as such reference.

[0041] For example, in certain instances, expression of the PCSK9 gene is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. In some embodiment, the PCSK9 gene is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, the PCSK9 gene is suppressed by at least about 85%, 90%, or 95% by administration

of the double-stranded oligonucleotide of the invention. Tables 1, 2, provides a wide range of values for inhibition of expression obtained in an *in vitro* assay using various PCSK9 dsRNA molecules at various concentrations.

[0042] As used herein in the context of PCSK9 expression, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes which can be mediated by down regulating PCSK9 gene. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes which can be mediated by down regulating the PCSK9 genre), the terms "treat", "treatment", and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition. For example, in the context of hyperlipidemia, treatment will involve a decrease in serum lipid levels.

[0043] As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes which can be mediated by down regulating the PCSK9 gene on or an overt symptom of pathological processes which can be mediated by down regulating the PCSK9 gene. The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner, and may vary depending on factors known in the art, such as, e.g. the type of pathological processes which can be mediated by down regulating the PCSK9 gene, the patient's history and age, the stage of pathological processes which can be mediated by down regulating PCSK9 gene expression, and the administration of other anti-pathological processes which can be mediated by down regulating PCSK9 gene expression.

[0044] As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

[0045] The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof and are described in more detail below. The term specifically excludes cell culture medium.

[0046] As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

II. Double-stranded ribonucleic acid (dsRNA)

[0047] In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the PCSK9 gene in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of the PCSK9 gene, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein said dsRNA, upon contact with a cell expressing said PCSK9 gene, inhibits the expression of said PCSK9 gene by at least 40%. The dsRNA comprises two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) comprises a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of the PCSK9 gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s). The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, the PCSK9 gene is the human PCSK9 gene. Sequences of sense and antisense strand are as defined above.

[0048] The skilled person is well aware that dsRNAs comprising a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 1 and 2, the dsRNAs of the invention can comprise at least one strand of a length of minimally 21 nt. It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Tables 1 and 2 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs comprising a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 1 and 2, and differing in their ability to inhibit the expression of the PCSK9 gene in a FACS assay as described herein below by not more than

5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further dsRNAs that cleave within the target sequence provided in Tables 1 and 2 can readily be made using the PCSK9 sequence and the target sequence provided.

[0049] In addition, the RNAi agents provided in Tables 1 and 2 identify a site in the PCSK9 mRNA that is susceptible to RNAi based cleavage. Described are further RNAi agents that target within the sequence targeted by one of the agents of the present invention. As used herein a second RNAi agent is said to target within the sequence of a first RNAi agent if the second RNAi agent cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first RNAi agent. Such a second agent will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Tables 1 and 2 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the PCSK9 gene. For example, the last 15 nucleotides of SEQ ID NO: 1 (minus the added AA sequences) combined with the next 6 nucleotides from the target PCSK9 gene produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Tables 1 and 2.

[0050] Described is also a dsRNA which can contain one or more mismatches to the target sequence. The dsRNA of the invention contains no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5 or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of the PCSK9 gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of the PCSK9 gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of the PCSK9 gene is important, especially if the particular region of complementarity in the PCSK9 gene is known to have polymorphic sequence variation within the population.

[0051] At least one end of the dsRNA may have a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its overall stability. dsRNA having only one overhang has proven particularly stable and effective *in vivo*, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA may also have a blunt end, generally located at the 5'-end of the antisense strand. Such dsRNAs have improved stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. One or more of the nucleotides in the overhang may be replaced with a nucleoside thiophosphate.

[0052] The dsRNA may be chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Edrs.), John Whiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Chemical modifications may include, but are not limited to 2' modifications, modifications at other sites of the sugar or base of an oligonucleotide, introduction of non-natural bases into the olibonucleotide chain, covalent attachment to a ligand or chemical moiety, and replacement of internucleotide phosphate linkages with alternate linkages such as thiophosphates. More than one such modification may be employed.

[0053] Chemical linking of the two separate dsRNA strands may be achieved by any of a variety of well-known techniques, for example by introducing covalent, ionic or hydrogen bonds; hydrophobic interactions, van der Waals or stacking interactions; by means of metal-ion coordination, or through use of purine analogues. Generally, the chemical groups that can be used to modify the dsRNA include, without limitation, methylene blue; bifunctional groups, generally bis-(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; and psoralen. The linker may be a hexa-ethylene glycol linker. In this case, the dsRNA are produced by solid phase synthesis and the hexa-ethylene glycol linker is incorporated according to standard methods (e.g., Williams, D.J., and K.B. Hall, Biochem. (1996) 35:14665-14670). In a particular embodiment, the 5'-end of the antisense strand and the 3'-end of the sense strand are chemically linked via a hexaethylene glycol linker. At least one nucleotide of the dsRNA may comprise a phosphorothioate or phosphorodithioate groups. The chemical bond at the ends of the dsRNA is generally formed by triple-helix bonds.

[0054] The nucleotides at one or both of the two single strands may be modified to prevent or inhibit the degradation activities of cellular enzymes, such as, for example, without limitation, certain nucleases. Techniques for inhibiting the degradation activity of cellular enzymes against nucleic acids are known in the art including, but not limited to, 2'-amino modifications, 2'-amino sugar modifications, 2'-F sugar modifications, 2'-F modifications, 2'-alkyl sugar modifications, uncharged backbone modifications, morpholino modifications, 2'-O-methyl modifications, and phosphoramidate (see, e.g., Wagner, Nat. Med. (1995) 1:1116-8). Thus, at least one 2'-hydroxyl group of the nucleotides on a dsRNA is replaced by a chemical group, generally by a 2'-amino or a 2'-methyl group. Also, at least one nucleotide may be modified to form

a locked nucleotide. Such locked nucleotide contains a methylene bridge that connects the 2'-oxygen of ribose with the 4'-carbon of ribose. Oligonucleotides containing the locked nucleotide are described in Koshkin, A.A., et al., Tetrahedron (1998), 54: 3607-3630) and Obika, S. et al., Tetrahedron Lett. (1998), 39: 5401-5404). Introduction of a locked nucleotide into an oligonucleotide improves the affinity for complementary sequences and increases the melting temperature by several degrees (Braasch, D.A. and D.R. Corey, Chem. Biol. (2001), 8:1-7).

[0055] Conjugating a ligand to a dsRNA can enhance its cellular absorption as well as targeting to a particular tissue or uptake by specific types of cells such as liver cells. In certain instances, a hydrophobic ligand is conjugated to the dsRNA to facilitate direct permeation of the cellular membrane and or uptake across the liver cells. Alternatively, the ligand conjugated to the dsRNA is a substrate for receptor-mediated endocytosis. These approaches have been used to facilitate cell permeation of antisense oligonucleotides as well as dsRNA agents. For example, cholesterol has been conjugated to various antisense oligonucleotides resulting in compounds that are substantially more active compared to their non-conjugated analogs. See M. Manoharan Antisense & Nucleic Acid Drug Development 2002, 12, 103. Other lipophilic compounds that have been conjugated to oligonucleotides include 1-pyrene butyric acid, 1,3-bis-O-(hexadecyl)glycerol, and menthol. One example of a ligand for receptor-mediated endocytosis is folic acid. Folic acid enters the cell by folate-receptor-mediated endocytosis. dsRNA compounds bearing folic acid would be efficiently transported into the cell via the folate-receptor-mediated endocytosis. Li and coworkers report that attachment of folic acid to the 3'-terminus of an oligonucleotide resulted in an 8-fold increase in cellular uptake of the oligonucleotide. Li, S.; Deshmukh, H. M.; Huang, L. Pharm. Res. 1998, 15, 1540. Other ligands that have been conjugated to oligonucleotides include polyethylene glycols, carbohydrate clusters, cross-linking agents, porphyrin conjugates, delivery peptides and lipids such as cholesterol.

[0056] In certain instances, conjugation of a cationic ligand to oligonucleotides results in improved resistance to nucleases. Representative examples of cationic ligands are propylammonium and dimethylpropylammonium. Interestingly, antisense oligonucleotides were reported to retain their high binding affinity to mRNA when the cationic ligand was dispersed throughout the oligonucleotide. See M. Manoharan Antisense & Nucleic Acid Drug Development 2002, 12, 103 and references therein.

[0057] The ligand-conjugated dsRNA of the invention may be synthesized by the use of a dsRNA that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the dsRNA. This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the invention facilitate the synthesis of ligand-conjugated dsRNA by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates, optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the invention via reaction of a selected serum-binding ligand with a linking moiety located on the 5' position of a nucleoside or oligonucleotide. In certain instances, an dsRNA bearing an aralkyl ligand attached to the 3'-terminus of the dsRNA is prepared by first covalently attacking a monomer building block to a controlled-pore-glass support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

[0058] The dsRNAs used in the conjugates of the invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

[0059] Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. Nos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphorus linkages; U.S. Pat. Nos. 5.378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,386,023, drawn to backbone-modified oligonucleotides and the preparation thereof through reductive coupling; U.S. Pat. No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Pat. No. 5,459,255, drawn to modified nucleobases based on N-2 substituted purines; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. Nos. 5,587,361 and 5,599,797, drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat.

No. 5,223,168, and U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. Nos. 5,602,240, and 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. Nos. 6,262,241, and 5,459,255, drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

[0060] In the ligand-conjugated dsRNA and ligand-molecule bearing sequence-specific linked nucleosides of the invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

[0061] When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. Oligonucleotide conjugates bearing a variety of molecules such as steroids, vitamins, lipids and reporter molecules, has previously been described (see Manoharan et al., PCT Application WO 93/07883). In a preferred embodiment, the oligonucleotides or linked nucleosides of the invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

[0062] The incorporation of a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-allyl, 2'-O-aminoalkyl or 2'-deoxy-2'-fluoro group in nucleosides of an oligonucleotide confers enhanced hybridization properties to the oligonucleotide. Further, oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Thus, functionalized, linked nucleosides of the invention can be augmented to include either or both a phosphorothioate backbone or a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-aminoalkyl, 2'-O-allyl or 2'-deoxy-2'-fluoro group. A summary listing of some of the oligonucleotide modifications known in the art is found at, for example, PCT Publication WO 200370918.

[0063] In some embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5'-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5'-positions through a linking group. The amino group at the 5'-terminus can be prepared utilizing a 5'-Amino-Modifier C6 reagent. In one embodiment, ligand molecules may be conjugated to oligonucleotides at the 5'-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5'-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5'-terminus.

[0064] Examples of modified internucleoside linkages or backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' for 2'-5' to 5'-2'. Various salts, mixed salts and free-acid forms are also included.

[0065] Representative United States Patents relating to the preparation of the above phosphorus-atom-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; and 5,697,248, each of which is herein incorporated by reference.

[0066] Examples of modified internucleoside linkages or backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

[0067] Representative United States patents relating to the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360, 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

[0068] In certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand

molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such oligonucleotide conjugates have been listed above. Typical conjugation protocols involve the synthesis of oligonucleotides bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate. The use of a cholesterol conjugate is particularly preferred since such a moiety can increase targeting liver cells cells, a site of PCSK9 expression.

Vector encoded RNAi agents

[0069] The dsRNA of the invention can also be expressed from recombinant viral vectors intracellularly *in vivo.* The recombinant viral vectors of the invention comprise sequences encoding the dsRNA of the invention and any suitable promoter for expressing the dsRNA sequences. Suitable promoters include, for example, the U6 or H1 RNA pol III promoter sequences and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the dsRNA in a particular tissue or in a particular intracellular environment. The use of recombinant viral vectors to deliver dsRNA of the invention to cells *in vivo* is discussed in more detail below.

[0070] dsRNA of the invention can be expressed from a recombinant viral vector either as two separate, complementary RNA molecules, or as a single RNA molecule with two complementary regions.

[0071] Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (e.g, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

[0072] For example, lentiviral vectors of the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors of the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, e.g., Rabinowitz J E et al. (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

[0073] Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A et al., Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

[0074] Preferred viral vectors are those derived from AV and AAV. In a particularly preferred embodiment, the dsRNA of the invention is expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector comprising, for example, either the U6 or III RNA promoters, or the cytomegalovirus (CMV) promoter.

[0075] A suitable AV vector for expressing the dsRNA of the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010.

[0076] Suitable AAV vectors for expressing the dsRNA of the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent

Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

III. Pharmaceutical compositions comprising dsRNA

[0077] In one embodiment, the invention provides pharmaceutical compositions comprising a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the dsRNA is useful for treating a disease or disorder associated with the expression or activity of the PCSK9 gene, such as pathological processes which can be mediated by down regulating PCSK9 gene expression, sucxh as hyperlipidemia. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for delivery to the liver via parenteral delivery.

[0078] The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of the PCSK9 gene. The present inventors have found that, because of their improved efficiency, compositions comprising the dsRNA of the invention can be administered at surprisingly low dosages. A dosage of 5 mg dsRNA per kilogram body weight of recipient per day is sufficient to inhibit or suppress expression of the PCSK9 gene and may be administered systemically to the patient.

[0079] In general, a suitable dose of dsRNA will be in the range of 0.01 to 5.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 microgram to 1 mg per kilogram body weight per day. The pharmaceutical composition may be administered once daily, or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art.

[0080] The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

[0081] Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes which can be mediated by down regulating PCSK9 gene expression. Such models are used for *in vivo* testing of dsRNA, as well as for determining a therapeutically effective dose.

[0082] Any method can be used to administer a dsRNA of the present invention to a mammal. For example, administration can be direct; oral; or parenteral (e.g., by subcutaneous, intraventricular, intramuscular, or intraperitoneal injection, or by intravenous drip). Administration can be rapid (e.g., by injection), or can occur over a period of time (e.g., by slow infusion or administration of slow release formulations).

[0083] Typically, when treating a mammal with hyperlipidemia, the dsRNA molecules are administered systemically via parental means. For example, dsRNAs, conjugated or unconjugate or formulated with or without liposomes, can be administered intravenously to a patient. For such, a dsRNA molecule can be formulated into compositions such as sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions in liquid or solid oil bases. Such solutions also can contain buffers, diluents, and other suitable additives. For parenteral, intrathecal, or intraventricular administration, a dsRNA molecule can be formulated into compositions such as sterile aqueous solutions, which also can contain buffers, diluents, and other suitable additives (e.g., penetration enhancers, carrier compounds, and other pharmaceutically acceptable carriers).

[0084] In addition, dsRNA molecules can be administered to a mammal as biologic or abiologic means as described in, for example, U.S. Pat. No. 6,271,359. Abiologic delivery can be accomplished by a variety of methods including, without limitation, (1) loading liposomes with a dsRNA acid molecule provided herein and (2) complexing a dsRNA molecule with lipids or liposomes to form nucleic acid-lipid or nucleic acid-liposome complexes. The liposome can be composed of cationic and neutral lipids commonly used to transfect cells *in vitro.* Cationic lipids can complex (e.g., charge-associate) with negatively charged nucleic acids to form liposomes. Examples of cationic liposomes include, without limitation, lipofectin, lipofectamine, lipofectace, and DOTAP. Procedures for forming liposomes are well known in the art. Liposome compositions can be formed, for example, from phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, or dioleoyl phosphatidylethanolamine. Numerous lipophilic agents are commercially available, including Lipofectin.RTM. (Invitrogen/Life Technologies, Carlsbad, Calif.) and Effectene.TM. (Qiagen, Valencia, Calif.). In addition, systemic delivery methods can be optimized using commercially available cationic lipids such as DDAB or DOTAP, each of which can be mixed with a neutral lipid such as DOPE or cholesterol. In some cases, liposomes such as those described by Templeton et al. (Nature Biotechnology, 15: 647-652 (1997)) can be used. In other embodiments, polycations such as polyethyleneimine can be used to achieve delivery *in*

vivo and ex vivo (Boletta et al., J. Am Soc. Nephrol. 7: 1728 (1996)). Additional information regarding the use of liposomes to deliver nucleic acids can be found in U.S. Pat. No. 6,271,359, PCT Publication WO 96/40964 and Morrissey, D. et al. 2005. Nat Biotechnol. 23(8):1002-7.

[0085] Biologic delivery can be accomplished by a variety of methods including, without limitation, the use of viral vectors. For example, viral vectors (e.g., a denovirus and herpesvirus vectors) can be used to deliver dsRNA molecules to liver cells. Standard molecular biology techniques can be used to introduce one or more of the dsRNAs provided herein into one of the many different viral vectors previously developed to deliver nucleic acid to cells. These resulting viral vectors can be used to deliver the one or more dsRNAs to cells by, for example, infection.

[0086] dsRNAs of the present invention can be formulated in a pharmaceutically acceptable carrier or diluent. A "pharmaceutically acceptable carrier" (also referred to herein as an "excipient") is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle. Pharmaceutically acceptable carriers can be liquid or solid, and can be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties. Typical pharmaceutically acceptable carriers include, by way of example and not limitation: water; saline solution; binding agents (e.g., polyinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose and other sugars, gelatin, or calcium sulfate); lubricants (e.g., starch, polyethylene glycol, or sodium acetate); disintegrates (e.g., starch or sodium starch glycolate); and wetting agents (e.g., sodium lauryl sulfate).

[0087] In addition, dsRNA that target the PCSK9 gene can be formulated into compositions containing the dsRNA admixed, encapsulated, conjugated, or otherwise associated with other molecules, molecular structures, or mixtures of nucleic acids. For example, a composition containing one or more dsRNA agents that target the PCSK9 gene can contain other therapeutic agents such as othr lipid lowering agents (e.g., statins).

Methods for treating diseases that can be modulated by down regulating the expression of PCSK9

[0088] The methods and compositions described herein can be used to treat diseases and conditions that can be modulated by down regulating PCSK9 gene expression. For example, the compositions described herein can be used to treat hyperlipidemia and other forms of lipid inbalance such as hypercholesterolemia, hypertriglyceridemia and the pathological conditions associated with thiese disorders such as heart and circulatory diseases.

Methods for inhibiting expression of the PCSK9 gene

[0089] Described is also a method for inhibiting the expression of the PCSK9 gene in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target PCSK9 gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of the target PCSK9 gene. Compositions and methods for inhibiting the expression of these PCSK9 genes using dsRNAs can be performed as described elsewhere herein.

[0090] The method may comprise administering a composition comprising a dsRNA, wherein the dsRNAs comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of the PCSK9 gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol) administration. In preferred embodiments, the compositions are administered by intravenous infusion or injection.

[0091] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Gene Walking of the PCSK9 gene

[0092] siRNA design was carried out to identify in two separate selections

a) siRNAs targeting PCSK9 human and either mouse or rat mRNA and

b) all human reactive siRNAs with predicted specificity to the target gene PCSK9.

[0093] mRNA sequences to human, mouse and rat PCSK9 were used: Human sequence NM_174936.2 was used as

reference sequence during the complete siRNA selection procedure.

[0094] 19 mer stretches conserved in human and mouse, and human and rat PCSK9 mRNA sequences were identified in the first step, resulting in the selection of siRNAs crossreactive to human and mouse, and siRNAs crossreactive to human and rat targets

[0095] SiRNAs specifically targeting human PCSK9 were identified in a second selection. All potential 19mer sequences of human PCSK9 were extracted and defined as candidate target sequences. Sequences cross-reactive to human, monkey, and those cross-reactive to mouse, rat, human and monkey are all listed in Tables 1 and 2. Chemically modified versions of those sequences and their activity in both *in vitro* and *in vivo* assays are also listed in tables 1 and 2 and examples given in Figures 2-8.

[0096] In order to rank candidate target sequences and their corresponding siRNAs and select appropriate ones, their predicted potential for interacting with irrelevant targets (off-target potential) was taken as a ranking parameter. siRNAs with low off-target potential were defined as preferable and assumed to be more specific *in vivo.*

[0097] For predicting siRNA-specific off-target potential, the following assumptions were made:

1) positions 2 to 9 (counting 5' to 3') of a strand (seed region) may contribute more to off-target potential than rest of sequence (non-seed and cleavage site region)

2) positions 10 and 11 (counting 5' to 3') of a strand (cleavage site region) may contribute more to off-target potential than non-seed region

3) positions 1 and 19 of each strand are not relevant for off-target interactions

4) an off-target score can be calculated for each gene and each strand, based on complementarity of siRNA strand sequence to the gene's sequence and position of mismatches

5) number of predicted off-targets as well as highest off-target score must be considered for off-target potential

6) off-target scores are to be considered more relevant for off-target potential than numbers of off-targets

7) assuming potential abortion of sense strand activity by internal modifications introduced, only off-target potential of antisense strand will be relevant

[0098] To identify potential off-target genes, 19me candidate sequences were subjected to a homology search against publically available human mRNA sequences.

[0099] The following off-target properties for each 19mer input sequence were extracted for each off-target gene to calculate the off-target score:

Number of mismatches in non-seed region

Number of mismatches in seed region

Number of mismatches in cleavage site region

[0100] The off-target score was calculated for considering assumption 1 to 3 as follows:

$$\text{Off-target score} = \text{number of seed mismatches} * 10 + \text{number of cleavage site mismatches} * 1.2 + \text{number of non-seed mismatches} * 1$$

[0101] The most relevant off-target gene for each siRNA corresponding to the input 19mer sequence was defined as the gene with the lowest off-target score. Accordingly, the lowest off-target score was defined as the relevant off-target score for each siRNAs.

dsRNA synthesis

Source of reagents

[0102] Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA synthesis

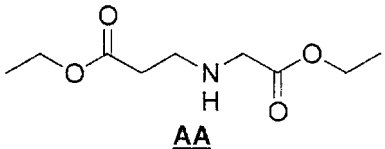
[0103] Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 pmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH. Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing 2'-O-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and 2'-O-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

[0104] Deprotection and purification of the crude oligoribonueleotides by anion exchange 1-IPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at -20 °C until use.

[0105] For the synthesis of 3'-cholesterol-conjugated siRNAs (herein referred to as -Chol-3'), an appropriately modified solid support was used for RNA synthesis. The modified solid support was prepared as follows:

Diethyl-2-azabutane-1,4-dicarboxylate **AA**

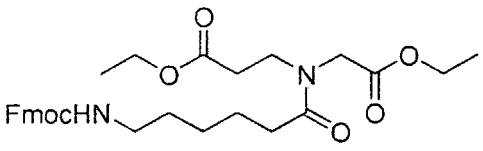
[0106]



AA

[0107] A 4.7 M aqueous solution of sodium hydroxide (50 mL) was added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) was added and the mixture was stirred at room temperature until completion of the reaction was ascertained by TLC. After 19 h the solution was partitioned with dichloromethane (3 x 100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated. The residue was distilled to afford AA (28.8 g, 61%).

[0108] 3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**



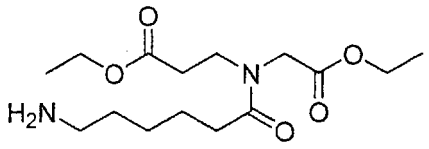
AB

[0109] Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) was added to the solution at 0°C. It was then followed by

the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution was brought to room temperature and stirred further for 6 h. Completion of the reaction was ascertained by TLC. The reaction mixture was concentrated under vacuum and ethyl acetate was added to precipitate diisopropyl urea. The suspension was filtered. The filtrate was washed with 5% aqueous hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer was dried over sodium sulfate and concentrated to give the crude product which was purified by column chromatography (50 % EtOAC/Hexanes) to yield 11.87 g (88%) of AB,

3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**

[0110]

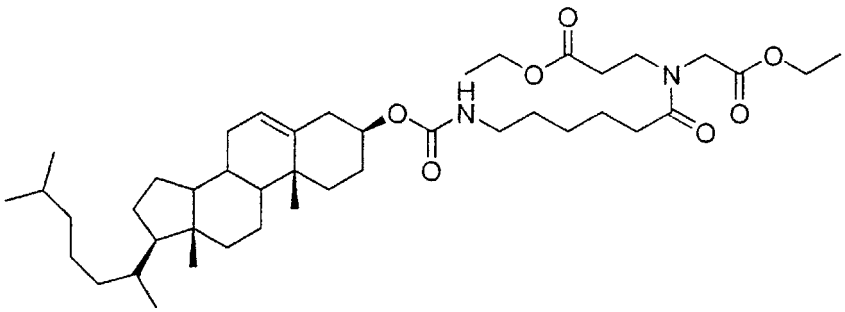


AC

[0111] 3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) was dissolved in 20% piperidine in dimethylformamide at 0°C. The solution was continued stirring for 1 h. The reaction mixture was concentrated under vacuum, water was added to the residue, and the product was extracted with ethyl acetate. The crude product was purified by conversion into its hydrochloride salt.

3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**

[0112]

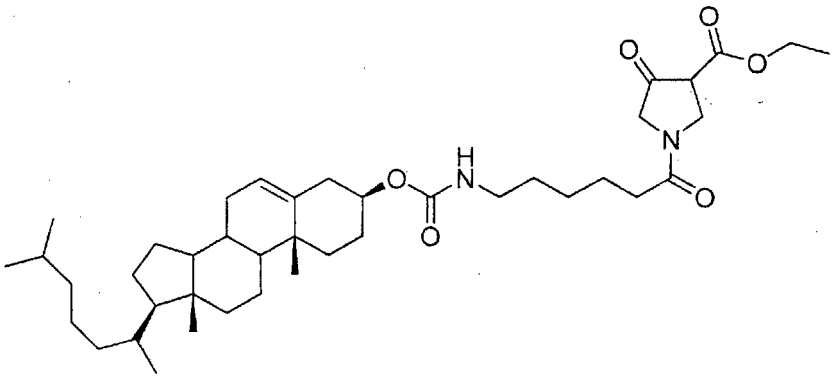


AD

[0113] The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester AC (4.7 g, 14.8 mmol) was taken up in dichloromethane. The suspension was cooled to 0°C on ice. To the suspension diisopropylethylamine (3.87 g, 5.2 mL, 30 mmol) was added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was diluted with dichloromethane and washed with 10% hydrochloric acid. The product was purified by flash chromatography (10.3 g, 92%).

1-{6-17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**

[0114]

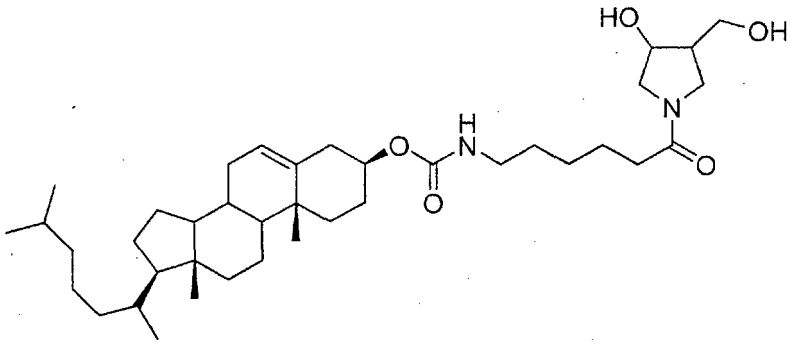


AE

[0115] Potassium t-butoxide (1.1 g, 9.8 mmol) was slurried in 30 mL of dry toluene. The mixture was cooled to 0°C on ice and g (6.6 mmol) of diester AD was added slowly with stirring within 20 mins. The temperature was kept below 5°C during the addition. The stirring was continued for 30 mins at 0°C and 1 mL of glacial acetic acid was added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture was extracted twice with 100 mL of dichloromethane each and the combined organic extracts were washed twice with 1.0 mL of phosphate buffer each, dried, and evaporated to dryness. The residue was dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts were adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which were combined, dried and evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**

[0116]

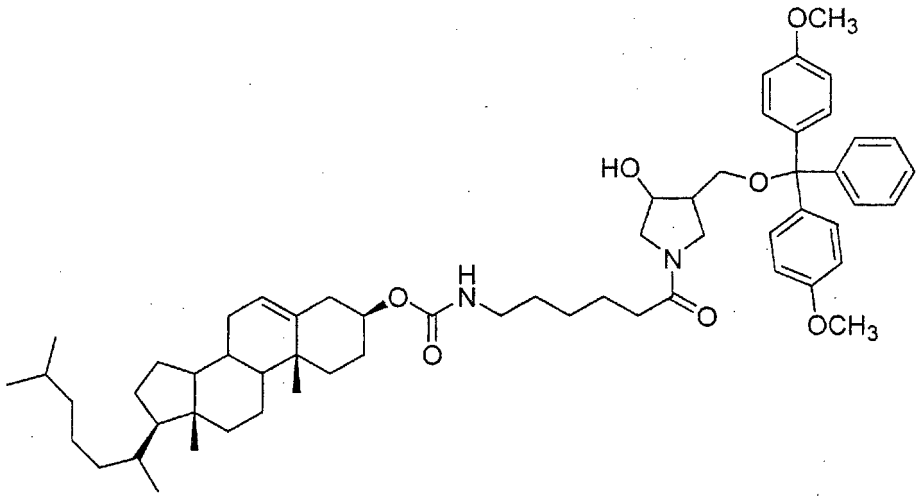


AF

[0117] Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.3 mL) was added, the mixture was extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer was dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which was purified by column chromatograph (10% MeOH/$CHCl_3$) (89%).

(6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**

[0118]

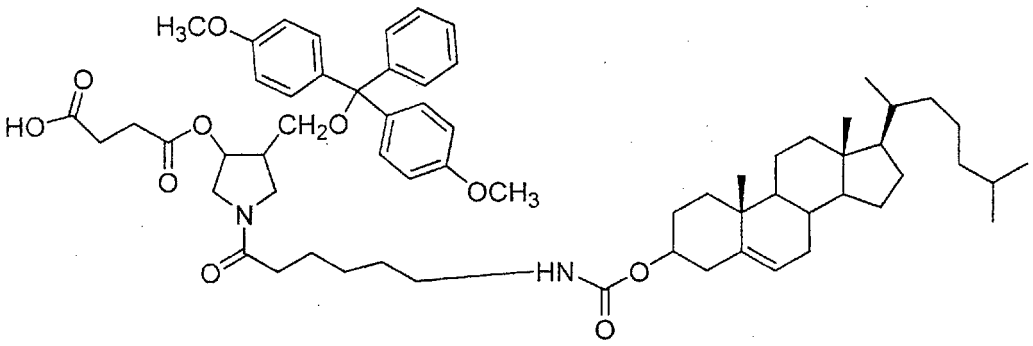


AG

[0119] Diol AF (1.25 gm 1.9.94 mmol) was dried by evaporating with pyridine (2 x 5 mL) *in* vacuo. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) were added with stirring. The reaction was carried out at room temperature overnight. The reaction was quenched by the addition of methanol. The reaction mixture was concentrated under vacuum and to the residue dichloromethane (50 mL) was added. The organic layer was washed with 1 M aqueous sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine was removed by evaporating with toluene. The crude product was purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOR/$CHCl_3$) (1.73 g, 95%).

Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-pyrrolidin-3-yl) ester **AH**

[0120]

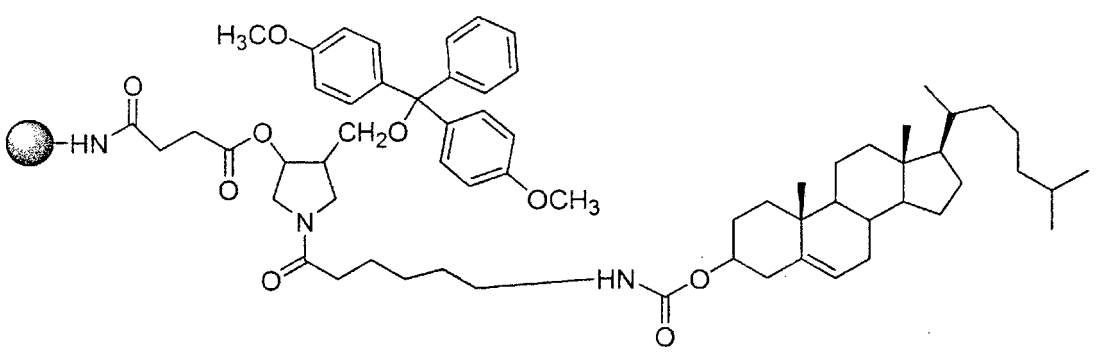


AH

[0121] Compound AG (1.0 g, 1.05 mmol) was mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture was dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) was added and the solution was stirred at room temperature under argon atmosphere for 16 h. It was then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was used as such for the next step.

Cholesterol derivatised CPG **AI**

[0122]



AI

[0123] Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242 mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) were added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) was added. The reaction mixture turned bright orange in color. The solution was agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) was added. The suspension was agitated for 2 h. The CPG was filtered through a sintered funnel and washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups were masked using acetic anhydride/pyridine. The achieved loading of the CPG was measured by taking UV measurement (37 mM/g).

[0124] The synthesis of siRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") was performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step was performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

[0125] Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 1-2.

Table 1-2: Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation [a]	Nucleotide(s)
A, a	2'-deoxy-adenosine-5'-phosphate, adenosine-5'-phosphate
C, c	2'-deoxy-cytidine-5'-phosphate, cytidine-5'-phosphate
G, g	2'-deoxy-guanosine-5'-phosphate, guanosine-5'-phosphate
T, t	2'-deoxy-thymidine-5'-phosphate, thymidine-5-phosphate
U, u	2'-deoxy-uridine-5'-phosphate, uridine-5'-phosphate
N, n	any 2'-deoxy-nucleotide/nucleotide (G, A, C, or T, g, a, c or u)
Am	2'-*O*-methyladenosine-5'-phosphate
Cm	2'-*O*-methylcytidine-5'-phosphate
Gm	2'-*O*-methylguanosine-5'-phosphate
Tm	2'-*O*-methyl-thymidine-5'-phosphate
Um	2'-*O*-methyleiridine-5'-phosphate
Af	2'-fluoro-2'-deoxy-adenosine-5' -phosphate
Cf	2'-fluoro-2'-deoxy-cytidine-5'-phosphate

(continued)

Abbreviation [a]	Nucleotide(s)
Gf	2'-fluoro-2'-deoxy-guanosine-5'-phosphate
Tf	2'-fluoro-2'-deoxy-thymidine-5'-phosphate
Uf	2'-fluoro-2'-deoxy-uridine-5'-phosphate
A, C, G, T, U, a, c, g, t, u	underlined: nucleoside-5'-phosphorothioate
am, cm, gm, tm, um	underlined: 2-*O*-methyl-nucleoside-5'-phosphorothioate
[a]capital letters represent 2'-deoxyribonucleotides (DNA), lower case letters represent ribonucleotides (RNA)	

PCSK9 siRNAs screening in HuH7 , HepG2, Hela and Primary Monkey Hepatocytes Discovers Highly Active Sequences

[0126] HuH-7cells were obtained from JCRB Cell Bank (Japanese Collection of Research Bioresources) (Shinjuku, Japan, cat. No.: JCRB0403) Cells were cultured in Dulbecco's MEM (Biochrom AG, Berlin, Germany, cat. No. F0435) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany, cat. No. S0115), Penicillin 100 U/ml. Streptomycin 100 μg/m) (Biochrom AG, Berlin, Germany, cat. No. A2213) and 2mM L-Glutamin (Biochrom AG. Berlin, Germany, cat. No K0282) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany). HepG2 and Hela cells were obtained from American Type Culture Collection (Rockville, MD, cat. No. B-8065) and cultured in MEM (Gibco Invitrogen. Karlsruhe, Germany, cat. No. 21090-022) supplemented to contain 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germans, cat. No. S0115), Penicillin 100 U/ml, Streptomycin 100 μg/ml (Biochrom AG, Berlin, Germany, cat. No. A2213), 1x Non Essential Amino Acids (Biochrom AG, Berlin, Germany, cat. No. K-0293), and 1mM Sodium Pyruvate (Biochrom AG, Berlin, Germany, cat. No. L-0473) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Kendro Laboratory Products, Langenselbold, Germany).

[0127] For transfection with siRNAs. HuH7, HepG2, or Hela cells were seeded at a density of 2.0 x 10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (30nM for single dose screen) was carried out with lipofectamine 2000 (Invitrogen GmbH. Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer.

[0128] 24 hours after transfection HuH7 and HepG2 cells were lysed and PCSK9 mRNA levels were quantified with the Quantigene Explore Kit (Genosprectra, Dumbarton Circle Fremont, USA, cat. No. QG-000-02) according to the protocol. PCSK9 mRNA levels were normalized to GAP-DH mRNA. For each siRNA eight individual datapoints were collected. siRNA duplexes unrelated to PCSK9 gene were used as control. The activity of a given PCSK9 specific siRNA duplex was expressed as percent PCSK9 mRNA concentration in treated cells relative to PCSK9 mRNA concentration in cells treated with the control siRNA duplex.

[0129] Primary cynomolgus monkey hepatocytes (cryopreserved) were obtained from *In vitro* Technologies, Inc. (Baltimore, Maryland, USA, cat No M00305) and cultured in InVitroGRO CP Medium (cat No Z99029) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

[0130] For transfection with siRNA, primary cynomolgus monkey cells were seeded on Collagen coated plates (Fisher Scientific, cat. No. 08-774-5) at a density of 3.5 x 10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (eight 2-fold dilution series starting from 30nM) in duplicates was carried out with lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer.

[0131] 16 hours after transfection medium was changed to fresh InVitroGRO CP Medium with Torpedo Antibiotic Mix (*In vitro* Technologies, Inc. cat. No Z99000) added.

[0132] 24 hours after medium change primary cynomolgus monkey cells were lysed and PCSK9 mRNA levels were quantified with the Quantigene Explore Kit (Genosprectra, Dumbarton Circle Fremont, USA, cat. No. QG-000-02) according to the protocol. PCSK9 mRNA levels were normalized to GAPDH mRNA. Normalized PCSK9/GAPDH ratios were then compared to PCSK9/GAPDH ratio of lipofectamine 2000 only control.

[0133] Tables 1-2 (and Figure 6) summarize the results and provides examples of *in vitro* screens in different cell lines at different doses. Silencing of PCSK9 transcript was expressed as percentage of remaining transcript at a given dose. Highly active sequences are those with less than 70% transcript remaining post treatment with a given siRNA at a dose less than or equal to 100nm. Very active sequences are those that have less than 60% of transcript remaining after treatment with a dose.less than or equal to 100nM. Active sequences are those that have less than 85% transcript remaining after treatment with a high dose (100nM). Examples of active siRNAs were also screened *in vivo* in mouse in lipidoid formulations as described below. Active sequences *in vitro* were also generally active *in vivo* (See figure Figure 6 example).

***In vivo* Efficacy Screen of PCSK9 siRNAs**

Formulation Procedure

[0134] The lipidoid LNP-01 ·4HCl (MW 1487) (Figure 1), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) were used to prepare lipid-siRNA nanoparticles. Stock solutions of each in ethanol were prepared: LNP-01, 133 mg/mL; Cholesterol, 25 mg/mL, PEG-Ceramide C16, 100 mg/mL. LNP-01, Cholesterol, and PEG-Ceramide C16 stock solutions were then combined in a 42:48:10 molar ratio. Combined lipid solution was mixed rapidly with aqueous siRNA (in sodium acetate pH 5) such that the final ethanol concentration was 35-45% and the final sodium acetate concentration was 100-300 mM. Lipid-siRNA nanoparticles formed spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture was in some cases extruded through a polycarbonate membrane (100 nm cut-off) using a thermobarrel extruder (Lipex Extruder, Northern Lipids, Inc). In other cases, the extrusion step was omitted. Ethanol removal and simultaneous buffer exchange was accomplished by either dialysis or tangential flow filtration. Buffer was exchanged to phosphate buffered saline (PBS) pH 7.2.

Characterization of formulations

[0135] Formulations prepared by either the standard or extrusion-free method are characterized in a similar manner. Formulations are first characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles are measured by dynamic light scattering using a Malvem Zetasizer Nano ZS (Malvern, USA). Particles should be 20-300 nm, and ideally, 40-100 nm in size. The particle size distribution should be unimodal. The total siRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated siRNA is incubated with the RNA-binding dye Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, 0.5% Triton-X100. The total siRNA in the formulation is determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" siRNA content (as measured by the signal in the absence of surfactant) from the total siRNA content. Percent entrapped siRNA is typically >85%.

Bolus dosing

[0136] Bolus dosing of formulated siRNAs in C57/BL6 mice (5/group, 8-10 weeks old, Charles River Laboratories, MA) was performed by tail vein injection using a 27G needle. SiRNAs were formulated in LNP-01 (and then dialyzed against PBS) at 0.5 mg/ml concentration allowing the delivery of the 5mg/kg dose in 10 μl/g body weight. Mice were kept under an infrared lamp for approximately 3 min prior to dosing to case injection.

[0137] 48 hour post dosing mice were sacrificed by CO_2-asphyxiation. 0.2 ml blood was collected by retro-orbital bleeding and the liver was harvested and frozen in liquid nitrogen. Serum and livers were stored at -80°C.

[0138] Frozen livers were grinded using 6850 Freezer/Mill Cryogenic Grinder (SPEX CentriPrep, Inc) and powders stored at -80°C until analysis.

[0139] PCSK9 mRNA levels were detected using the branched-DNA technology based kit from QuantiGene Reagent System (Genospectra) according to the protocol. 10-20mg of frozen liver powders was lysed in 600 ul of 0.16 ug/ml Proteinase K (Epicentre, #MPRK092) in Tissue and Cell Lysis Solution (Epicentre, #MTC096H) at 65°C for 3hours. Then 10 ul of the lysates were added to 90ul of Lysis Working Reagent (1 volume of stock Lysis Mixture in two volumes of water) and incubated at 52°C overnight on Genospectra capture plates with probe sets specific to mouse PCSK9 and mouse GAPDH or cyclophilin B. Nucleic acid sequences for Capture Extender (CE), Label Extender (LE) and blocking (BL) probes were selected from the nucleic acid sequences of PCSK9, GAPDH and cyclophilin B with the help of the QuantiGenc ProbeDesigner Software 2.0 (Genospectra, Fremont, CA, USA, cat. No. QG-002-02). Chemo luminescence was read on a Victor2-Light (Perkin Elmer) as Relative light units. The ratio of PCSK9 mRNA to GAPDH or cyclophilin B mRNA in liver lysates was averaged over each treatment group and compared to a control group treated with PBS or a control group treated with an unrelated siRNA (blood coagulation factor VII).

[0140] Total serum cholesterol in mouse serum was measured using the StanBio Cholesterol LiquiColor kit (StanBio Laboratoriy, Boerne, Texas, USA) according to manufacturer's instructions. Measurements were taken on a Victor2 1420 Multilabel Counter (Perkin Elmer) at 495 nm.

Examples

[0141] 32 PCSK9 siRNAs formulated in LNP-01 liposomes were tested *in vivo* in a mouse model. The experiment was performed at 5mg/kg siRNA dose and at least 10 PCSK9 siRNAs showed more than 40% PCSK9 mRNA knock down compared to a control group treated with PBS, while control group treated with an unrelated siRNA (blood coag-

ulation factor VII) had no effect (Figures 2-5). Silencing of PCSK9 transcript also coorelated with a lowering of cholesterol in these animals (Figures 4-5). In addition there was a strong coorelation between those molecules that were active *in vitro* and those active *in vivo* (Figure 6). Sequences containing different chemical modifications were also screened *in vitro* (Tables 1 and 2) and *in vivo*. As an example, less modified sequences 9314 and 9318, and a more modified versions of that sequence 9314-(10792, 1079 3, and 10796); 9318-(10794, 10795, 10797) were tested both in *vitro* (In primary monkey hepatocytes) or *in vivo* (9314 and 10792) formulated in LNP-01. Figure 7 (also see Tables 1 and2) shows that the parent molecules 9314 and 9318 and the modified versions are all active *in vitro*. Figure 8 as an example shows that both the parent 9314 and the more highly modified 10792 sequences are active *in vivo* displaying 50-60% silencing of endogenous PCSK9 in mice. Figure 9 furthur exemplifies that activity of other chemically modified versions of the parents 9314 and 10792.

dsRNA expression vectors

[0142] In another aspect of the invention, PCSK9 specific dsRNA molecules that modulate PCSK9 gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., TIG. (1996), 12:5-10; Skillern, A., et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, US Pat. No. 6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., Proc. Natl. Acad. Sci. USA (1995) 92:1292).

[0143] The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

[0144] The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., Curr. Topics Micro. Immunol. (1992) 158:97-129)); adenovirus (see, for example, Berkner, et al., BioTechniques (1998) 6:616), Rosenfeld et al. (1991, Science 252:431-434), and Rosenfeld et al. (1992), Cell 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, e.g., Eglitis, et al., Science (1985) 230:1395-1398; Danos and Mulligan, Proc. Natl. Acad. Sci. USA (1998) 85:6460-6464; Wilson et al., 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al., 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber et al., 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al., 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al., 1991, Science 254:1802-1805; van Beusechem. et al., 1992, Proc. Nad. Acad. Sci. USA 89:7640-19 ; Kay et al., 1992, Human Gene Therapy 3:641-647; Dai et al., 1992, Proc. Natl.Acad. Sci. USA 89:10892-10895; Hwu et al., 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette et al., 1991, Human Gene Therapy 2:5-10; Cone et al., 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

[0145] The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U I snRNA promoter) or generally RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

[0146] In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1-thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

[0147] Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or

intraimuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

[0148] dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single PCSK9 gene or multiple PCSK9 genes over a period of a week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection, can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection, of ex vivo cells can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

[0149] The PCSK9 specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0150] Those skilled in the art are familiar with methods and compositions in addition to those specifically set out in the instant disclosure which will allow them to practice this invention to the full scope of the claims hereinafter appended.

Table 1.

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2-20	AGCGACGUC-GAGGCGCUG-CATT	1	UGAGCGCCUC GACGUCGCUT T	2	AD-15220				35		
15-33	CGCUCAUGG-UUGCAGGCG-GTT	3	CCGCCUG-CAACCAU-GAGCGTT	4	AD-15275				56		
16-34	GCUCAUGGU-UGCAGGCG-GGTT	5	CCCGCCUC-CAACCAU-CAGCTT	6	AD-15301				70		
30-48	GCG-GGCGCCGCCG UUCAGUTT	7	ACUGAACG-GCG-GCGCCCCTT	8	AD-15276				42		
31-49	CGGGCGCG-GCGCCGUU-CAGUUTT	9	AACUGAACG-GCG-GCGCCCGTT	10	AD-15302				32		
32-50	GGGCGCCGCC GUUCAGU-UCTT	11	GAACUUAACG-GCG-GCGCCCTT	12	AD-15303				37		
40-58	CCGUUCAGU-UCAG-GGUCUGTT	13	CAGACCCU-GAACUGAACG-GTT	14	AD-15221				30		
43-61	UUCAGUUCAG-GUCUGAGCTT	15	GCUCA-GAGCCCU-GAACUGAATT	16	AD-15413				61		
82-100	GUGAGACUG-GCUCGGGCG-GTT	17	CCGCCCGAGC CAGUCUCATT	18	AD-15304				70		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
100-118	GGCCG-GGACGCGUCG UUGCTT	19	GCAAC-GACGCGUC-CCUUCCTT	20	AD-15305				36		
101-119	GCCGACGCGU CCUUCCATT	21	UUCAAC-CACGCGUC-CCGCCTT	22	AD-15306				20		
102-120	CCG-GACGCGUCG-UUGCAGTT	23	CUGCAAC-GACGCGUC-CCCCTT	24	AD-15307				38		
105-123	GGACGCGUCG UUGCAG-CAGTT	25	CUGCUGCAAC-GACGCGUC-CTT	26	AD-15277				50		
135-153	UCCCAGCCAG-GAUUC-CGCCGTsT	27	CGCGGAAUC-CUGGCUG-GATsT	28	AD-9526	74	89				
135-153	ucccAGccAG-GAuuccGcGTsT	29	CGCGGAAUC-CUGGCUG-GGATsT	30	AD-9652		97				
136-154	CCCAGCCAG-GAUUC-CGCGCTsT	31	GCGCG-GAAUCCUG-GCUGGGTsT	32	AD-9519		78				
136-154	cccAGccAGGA-uuccGcGcTsT	33	GCGCG-GAAUCCUG-GCUGGGTsT	34	AD-9645		66				
138-156	CAGCCAGGAU-UCCGCGCGCT sT	35	GCGCGCG-GAAUCCUG-GCUGTsT	36	AD-9523		55				

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
138-156	cAGccAGGAu-uccGcGcGcTsT	37	GCGCGCG-GAAUCCUG-GCUGTsT	38	AD-9649		60				
185-203	AGCUCCUG-CACAGUCCUC-CTsT	39	GGAGGACU-GUGCAG-GAGCUTsT	40	AD-9569		112				
185-203	AGcuccuG-cAcAGuccuc-cTsT	41	GGAGGACU-GUGcAG-GAGCUTsT	42	AD-9695		102				
205-223	CACCGCAAG-GCU-CAAGCCGTT	43	CGCCUU-GAGCCU-UGCGGUGTT	44	AD-15222				75		
208-226	CGCAAGGCU-CAAG-GCGCCGTT	45	CGGCGCCUU-GAGCCU-UGCGTT	46	AD-15278				78		
210-228	CAAGGCU-CAAG-GCGCCGCCTT	47	GGCG-GCGCCUU-GAGCCUUGTT	48	AD-15178				83		
232-250	GUGGAC-CGCGCACG-GCCUCTT	49	GAG-GCCGUGCGCG GUCCACTT	50	AD-15308				84		
233-251	UGGACCGCG-CACGGCCU-CUTT	51	AGAG-GCCGUGCGCG GUCCATT	52	AD-15223				67		
234-252	GGACCGCG-CACGGCCU-CUAATT	53	UAGAG-GCCGUGCGCG GUCCTT	54	AD-15309				34		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
235-253	GACCGCG-CACGGCCU-CUAGTT	55	CUAGAG-GCCGUGCGCU GUCTT	56	AD-15279				44		
236-254	ACCGCG-CACGGCCU-CUAGGTT	57	CCUAGAG-GCCGUC-CGCCGUTT	58	AD-15194				63		
237-255	CCGCGCACG-GCCUCUAG-GUTT	59	ACCUAGAG-GCCGUGCGCG GTT	60	AD-15310				42		
238-256	CGCGCACG-GCCUCUAG-GUCTT	61	GACCUAGAG-GCCGUGCGCG TT	62	AD-15311				30		
239-257	GCGCACG-GCCUCUAG-GUCUTT	63	AGACCUAGAG-GCCGUGCTT	64	AD-15392				18		
240-258	CGCACG-GCCUCUAG-GUCUCTT	65	GAGACCUA-GAGCCCGUGC GTT	66	AD-15312				21		
248-266	CUCUAG-GUCUC-CUCGCCAGTT	67	CUGCCCAG-GACACCUA-GAGTT	68	AD-15313				19		
249-267	UCUAG-GUCUC-CUCGCCAG-GTT	69	CCUGGCGAG-GAGACCUA-GATT	70	AD-15280				81		
250-268	CUAGGUCUC-CUCGCCAG-GATT	71	UCCUG-GCGAGGA-GACCUAGTT	72	AD-15267				82		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
252-270	AGGUCUC-CUCGCCAG-GACATT	73	UGUCCUG-GCGAGGA-GACCUTT	74	AD-15314				32		
258-276	CCUCGCCAG-GACAGCAAC-CTT	75	GCUUGCU-GUCCUG-GCGAGGTT	76	AD-15315				74		
300-318	CGUCAGCUC-CAGGCGGUC-CTsT	77	GGAC-CGCCUG-GAGCU-GACGTsT	78	AD-9624		94				
300-318	cGucAGcuc-cAGGcGGuc-cTsT	79	GGAC-CGCCUG-GAGCU-GACGTsT	80	AD-9750		96				
301-319	GUCAGCUC-CAGGCGGUC-CUTsT	81	AGGAC-CGCCUG-GAGCUGACTsT	82	AD-9623	43	66				
301-319	GucAGcuccAG-GcGGuccuTsT	83	AGGAC-CGCCUG-GAGCUGACTsT	84	AD-9749		105				
370-388	GGCGCCCGUG CGCAGGAG-GTT	85	CCUCCUGCG-CACG-GGCGCCTT	86	AD-15384				48		
408-426	GGAGCUG-GUGCUAGCCU UGTsT	87	CAAGGCUAG-CACCAGCUC-CTsT	88	AD-9607		32	28		0,20	
408-426	GGAGcuG-GuGcuAGccu-uGTsT	89	cAAGGCuAG-cAGcAC-cAGCUCCTsT	90	AD-9733		78	73			

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
411-429	GCUG-GUGCUAGCCU UGCGUTsT	91	ACGCAAG-GCUAGCAC-CAGCTsT	92	AD-9524		23	28		0,07	
411-429	GcuG-GuGcuAGccu-uGcGuTsT	93	ACGcAAG-GCuAGcAC-cAGCTsT	94	AD-9650		91	90			
412-430	CUG-GUGCUAGCCU UGCGUUTsT	95	AACGCAAG-GCUAGCAC-CAGTsT	96	AD-9520		23	32			
412-430	CUG-GUGCUAGCCU UGCGUUTsT	97	AACCCAAC-CCUACCAC-CAGTsT	98	AD-9520		23				
412-430	cuG-GuGcuAGccu-uGcGuuTsT	99	AACGcAAG-GCuAGcAC-cAGTsT	100	AD-9646		97	108			
416-434	UGCUAGCCU-UGCGUUC-CGATsT	101	UCGGAACG-CAAGGCUAG-CATsT	102	AD-9608		37				
416-434	uGcuAGccu-uGcGuuc-cGATsT	103	UCGGAACG-cAAGGCuAG-cATsT	104	AD-9734		91				
419-437	UAGCCU-UGCGUUC-CGAGGATsT	105	UCCUCG-GAACGCAAG-GCUATsT	106	AD-9546		32				
419-437	uAGccuuGcGu-uccGAGGATsT	107	UCCUCG-GAACGcAAG-GCuATsT	108	AD-9672		57				

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
439-457	GACGGCCUG-GCCGAAG-CACTT	109	GUGCUUCG-GCCAG-GCCGUCTT	110	AD-15385				54		
447-465	GGCCGAAG-CACCCGAG-CACTT	111	GUGCUCG-GGUGCUUCG-GCCTT	112	AD-15393				31		
448-466	GCCGAAGCAC-CCGAG-CACGTT	113	CGUGCUCG-GGUGCUUCG-GCTT	114	AD-15316				37		
449-467	CCGAAGCAC-CCGAGCACG-GTT	115	CCGUGCUCG-GGUGCUUCG-GTT	116	AD-15317				37		
458-476	CCGAGCACG-GAAC-CACAGCTT	117	GCUGUGGU-UCCGUGCUCG GTT	118	AD-15318				63		
484-502	CAC-CGCUGCGCCA AGGAUCTT	119	GAUCCUUG-GCGCAGCG-GUGTT	120	AD-15195				45		
480-504	CCGCUGCGCC AAGGAUC-CGTT	121	CGGAUCCU-UGGCG-CAGCGGTT	122	AD-15224				57		
487-505	CGCUGCGCCA AGGAUCGUTT	123	ACGGAUCCU-UGGCG-CAGCGTT	124	AD-15188				42		
489-507	CUGCGCCAAG GAUCCGUG-GTT	125	CCACGGAUC-CUUGGCG-CAGTT	126	AD-15225				51		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
500-518	AUCCGUG-GAGGU-UGCCUGGTT	127	CCAGGCAAC-CUCCACG-GAUTT	128	AD-15281				89		
509-527	GGUUGCCUG-GCAC-CUACGUTT	129	ACGUAG-GUGCCAG-GCAACCTT	130	AD-15282				75		
542-560	AGGAGAC-CCACCUCUCG-CATT	131	UGCGAGAG-GUGGGUCUC-CUTT	132	AD-15319				61		
543-561	GGAGACCCAC-CUCUCG-CAGTT	133	CUGCGAGAG-GUGGGUCUC-CTT	134	AD-15226				56		
544-562	GAGACCCAC-CUCUCG-CAGUTT	135	ACUGCGA-GAGGUG-GGUCUCTT	136	AD-15271				25		
549-567	CCACCUCUCG-CAGUCA-GAGTT	137	CUCU-GACUGCGA-GAGGUGGTT	138	AD-15283				25		
552-570	CCUCUCG-CAGUCA-GAGCGCTT	139	GCGCUCU-GACUGCGA-GAGGTT	140	AD-15284				64		
553-571	CUCUCG-CAGUCA-GAGCGCATT	141	UGCGCUCU-GACUGCGA-GAGTT	142	AD-15189				17		
554-572	UCUCGCAGU-CAGAGCG-CACTT	143	GUGCGCUCU-GACUGCGATT	144	AD-15227				62		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
555-573	CUCGCAGUCA-GAGCG-CACUTsT	145	AGUGCGCU-CU-GACUGCGAGT sT	146	AD-9547		31	29		0,20	
555-573	cucGcAGucA-GAGcGcAcuTsT	147	AGUGCGCU-CU-GACUGCGAGT sT	148	AD-9673		56	57			
558-576	GCAGUCA-GAGCG-CACUGCCTsT	149	GGCAGUGCGC UCU-GACUGCTsT	150	AD-9548		54	60			
558-576	GcAGucA-GAGcG-cAcuGccTsT	151	GGcAGUGCGC UCU-GACUGCTsT	152	AD-9674		36	57			
606-624	GGGAUACCU-CACCAA-GAUCTsT	153	GAUCUUGGU-GAGGUAUC-CCTsT	154	AD-9529		60				
606-624	GGGAuAccu-cAccAA-GAucTsT	155	GAUCUUGGU-GAGGuAUC-CCTsT	156	AD-9655		140				
659-677	UGGUGAA-GAUGAGUG-GCGATsT	157	UCGCCACU-CAUCUUCAC-CATsT	158	AD-9605		27	31		0,27	
659-677	uGCuGAAGAu-GAGuG-GcGATsT	159	UCGCcACU-cAUCUUcAC-cATsT	160	AD-9731		31	31		0,32	

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
663-681	GAAGAU-GAGUG-GCGAC-CUGTsT	161	CAG-GUCGCCACU-CAUCUUCTsT	162	AD-9596		37				
663-681	GAAGAu-GAGuGGcGAc-cuGTsT	163	cAG-GUCGCcACU-cAUCUUCTsT	164	AD-9722		76				
704-722	CCCAUGUC-GACUACAUC-GATsT	165	UCGAU-GUAGUC-GACAUG-GGTsT	166	AD-9583		42				
704-722	cccAuGucGAc-uAcAucGATsT	167	UCGAU-GuAGUC-GAcAUGGGTsT	168	AD-9709		104				
718-736	AUCCAGGAG-GACUCCU-CUGTsT	169	CAGAG-GAGUCCUC-CUCGAUTsT	170	AD-9579		113				
718-736	AucGAGGAG-GAcuccu-cuGTsT	171	cAGAGGAGUC-CUCCUC-GAUTsT	172	AD-9705		81				
758-776	GGAACCUG-GAGCGGAU-UACTT	173	GUAAUC-CGCUCCACG-UUCCTT	174	AD-15394				32		
759-777	GAACCUG-GAGCGGAU-UACCTT	175	GGUAAUC-CGCUCCAGG-UUCTT	176	AD-15196				72		
760-778	AACCUG-GAGCGGAU-UACCCTT	177	GGGUAAUC-CGCUCCAGG-UUTT	178	AD-15197				85		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
777-795	CCCUCCACG-GUACCG-GGCGTT	179	CGCCCG-GUACCGUG-GAGGGTT	180	AD-15198				71		
782-800	CACGGUAC-CGGGCGGAU-GATsT	181	UCAUC-CGCCCG-GUAC-CGUGTsT	182	AD-9609	66	71				
782-800	cAcGGuAccG-GGcGGAu-GATsT	183	UcAUC-CGCCCGGuAC-CGUGTsT	184	AD-9735		115				
783-801	ACGGUACCG-GGCGGAU-GAATsT	185	UUCAUC-CGCCCG-GUACCGUTsT	186	AD-9537		145				
793-801	AcGGuAccG-GGcGGAu-GAATsT	187	UUcAUC-CGCCCGGuAC-CGUTsT	188	AD-9663		102				
784-802	CGGUACCG-GGCGGAU-GAAUTsT	189	AUUCAUC-CGCCCG-GUACCGTsT	190	AD-9528		113				
784-802	cGGuAccG-GGeGGAu-GAAuTsT	191	AUUcAUC-CGCCCGGuAC-CGTsT	192	AD-9654		107				
785-803	GGUACCG-GGCGGAU-GAAUATsT	193	UAUUCAUC-CGCCCG-GUACCTsT	194	AD-9515		49				
785-803	GCuAccG-GGcGGAu-GAAuATsT	195	uAUUcAUC-CGCCCGGuAC-CTsT	196	AD-9641		92				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
786-804	GUACCG-GGCGGAU-GAAUACTsT	197	GUAUUCAUC-CGCCCG-GUACTsT	198	AD-9514		57				
786-804	GuAccGGGcG-GAuGAAuAcTsT	199	GuAUUcAUC-CGCCCG-GuACTsT	200	AD-9640		89				
780-806	ACCGGGCG-GAUGAAUAC-CATsT	201	UGGUAUU-CAUC-CGCCCG-GUTsT	202	AD-9530		75				
788-806	AccGGGcG-GAuGAAuAc-cATsT	203	UGGuAUU-cAUCCGCCCG-GUTsT	204	AD-9656		77				
789-807	CCGGGCG-GAUGAAUAC-CAGTsT	205	CUGGUAUU-CAUC-CGCCCGGTsT	206	AD-9538	79	80				
789-807	ccGGGcGGAu-GAAuAccAGTsT	207	CUGCuAUU-cAUCCGCCCG-GTsT	208	AD-9664		53				
825-843	CCUGGUG-GAGGU-GUAUCUCTsT	209	GAGAUACAC-CUCCACCAG-GTsT	210	AD-9598	69	83				
825-843	ccuGGuGGAG-GuGuAucucTsT	211	GAGAuAcAC-CUCcACcAG-GTsT	212	AD-9724		127				
826-844	CUGGUGGAG-GUGUAUCUC-CTsT	213	GGAGAUACAC-CUCCAC-CAGTsT	214	AD-9625	58	88				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
826-844	cuGGuGGAG-GuGuAucuc-cTsT	215	GUAGAuAcAC-CUCcAC-cAGTsT	216	AD-9751		60				
827-845	UGGUGGAG-GUGUAUCUC-CUTsT	217	AGGA-GAUACACCUC-CACCATsT	218	AD-9556		46				
827-845	uGGuGGAGGu-GuAucuccuTsT	219	AGCA-GAuAcACCUC-cACcATsT	220	AD-9682		38				
828-846	GGUGGAGGU-GUAUCUCCU-ATsT	221	UAGGA-GAUACACCUC-CACCTsT	222	AD-9539	56	63				
828-846	GGuGGAGGu-GuAucuccuATsT	223	uAGGA-GAuAcACCUC-cACCTsT	224	AD-9665		83				
831-849	GGAGGU-GUAUCUCCUA-GACTsT	225	GUCUAGGA-GAUACACCUC-CTsT	226	AD-9517		36				
831-849	GGAGGu-GuAucuccuA-GAcTsT	227	GUCuAGCA-GAuAcACCUC-CTsT	228	AD-9643		40				
833-851	AGGU-GUAUCUCCUA-GACACTsT	229	GUGUCUAG-GAGAUACAC-CUTsT	230	AD-9610		36	34		0,04	
833-851	AGGuGuAucuc-cuAGAcAcTsT	231	GUGUCuAGGA-GAuAcAC-CCTsT	232	AD-9736		22	29		0,04	

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
833-851	AfgGfuGTu-AfuCfuCfcUfaG-faCfaCfTsT	233	p-gUfgUfcUfaGf-gAfgafuAfcAfc-CfuTsT	234	AD-14681				33		
833-851	AGGUfGU-fAUfCfUfCfCfUf-AGACfACfTsT	235	GUfGUfCfUfAG-GAGAUfAC-fACfCfUfTsT	236	AD-14691				27		
833-851	AgGuGuAuCuC-cUaGaCaCTsT	237	p-gUfgUfcUfaGf-gAfgAfuAfcAfc-CfuTsT	238	AD-14701				32		
833-851	AgGuGuAuCuC-cUaGaCaCTsT	239	GUfGUfCfUfAG-GAGAUfAC-fACfCfUfTsT	240	AD-14711				33		
833-851	AfgGfuGfuAfuC-fuCfcUfaGfaC-faCfTsT	241	CUCUCuaGGa-gAUACAccuTsT	242	AD-14721				22		
833-851	AGGUfGU-fAUfCfUfCfCfUf-AGACfACfTsT	243	GUGUCuaGGa-gAUACAccuTs	244	AD-14731				21		
833-851	AgGuCuAuCuC-cUaGaCaCTsT	245	GUGUCuaGGa-gAUACAccuTsT	246	AD-14741				22		
833-851	GfcAfcCf-cUfcAfuAfgGfc-CfuGfgAfTsT	247	p-uCfcAfgGfc-CfuAfuGfaGfg-GfuGfcTsT	248	AD-15087				37		
833-851	GCfACfCfCfUfC-fAUfAGGCfCfUf-GGATsT	249	UfCfCfAGGCfCf-UfAUfGAG-GGUfGCfTsT	2S0	AD-15097				51		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
833-851	GcAcCcU-cAuAgGcCuG-gATsT	251	p-uCfcAfgGfc-CfuAfuGf-aGfuGfcTsT	252	AD-15107				26		
833-851	CcAcCcU-cAuAgGcCuC-gATsT	253	UfCfAGGCfCfU-fAUfGAGGGUf-GCfTsT	254	AD-15117				28		
833-851	GfcAfcCf-cUfcAfuAfg-GfcGfuGfgAfTsT	255	UCCAGgcCUau-GAGGGugcTsT	256	AD-15127				33		
833-851	GCfACfCfCfUfC-fAUfAGGCfCfUf-GGATsT	257	UCCAGgcCUau-GAGGugcTsT	258	AD-15137				54		
833-851	GcAcCcU-cAuAgGcCuG-gATsT	259	UCCAGgcCUau-GAGGGugcTsT	260	AD-15147				52		
836-854	UCUAUCUC-CUAUACAC-CAGTsT	261	CUUGUCUAG-GAGCACATsT	262	AD-9516		94				
836-854	uGuAucuccuA-GAcAGTsT	263	CUGGUGCuAG-GAGAuAcATsT	264	AD-9642		105				
840-858	UCUC-CUACACAC-CAGCAUATsT	265	UAUGCUGGU-GUCUACCA-GATsT	266	AD-9562		46	51			
840-858	ucuccuAGAcAc-cAGcAuATsT	267	uAUUCUGUU-GUCuAGGA-GATsT	268	AD-9688		26	34		4,20	

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
840-858	UfcUfcCfuAf-sAfcCfaGf-cAfuAfTsT	269	p-uAfuGfcUfg-GfuGfuCfuAfg-GfaGfafsT	270	AD-14677				38		
840-858	UfCfUfCfCfUf-AGACfACfCf-AGCfAUfATsT	271	UfAUfGCfUfG-GUfGUfCfUf-AGATsT	272	AD-14687				52		
840-858	UcUcCuA-gAcAcCaG-cAuATsT	273	p-uAfuGfcUfg-GfuCfuAfgGfaG-faTsT	274	AD-14697				35		
840-858	UcUcCuA-gAcAcCaG-cAuATsT	275	UfAUfGCfUfG-GUfGUfCfUfAG-GAGATsT	276	AD-14707				58		
840-858	UfcUfcCfuAa-fAfcfcCfaGf-cAfuAfTst	277	UAUGCugGU-guCUAGGa-gaTsT	278	AD-14717				42		
840-858	UfCfUfCfCfUf-AGACfACfCf-AGCfAUfAATST	279	UAUGCugGU-guCUAGGa-gaTsT	280	AD-14727				50		
840-858	UcUcCuA-gAcAcCaU-cAuATsT	281	UAUGCugCU-guCUAGGa-gaTsT	282	AD-14737				32		
840-858	AfgGfc-CfuGfuGfgAfgU-fuUfaUfuCf-GfTsT	283	p-cCfgAfaUfAA-faCfuCfcAfgGfc-CfuTsT	284	AD-15083				16		
840-858	AGGCfCfUfG-GAGUfUfUdAU-fUfCfGGGTsT	285	CfCfGAAU-fAAACfUfCfCf-AGGCfCfUfTsT	286	AD-15093				24		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
840-958	AgGcCuGgAgU-uUaUuCgGTsT	287	p-cCfgAfaUfaA-faCfuCfcAfgGfc-CfuTs	288	AD-15103				11		
840-858	AgGcCuGgAgU-uUaUuCgGTsT	289	CfCfGAAU-fAAACfUfCfCf-AGGCfCfUfTsT	290	AD-15113				34		
840-858	AfgGfcCfcGfgAf-gUfuUfaUfuCfg-GfTsT	291	CCGAAuaAAcu CCAGGccuTsT	292	AD-15123				19		
840-858	AGGCfUfG-GAGUfUfUfAU-fUfCfCfGGTsT	293	CCGAAuaAAcu CCAGGccuTsT	294	AD-15133				15		
840-858	AgGcCuGgAgU-uUaUuCgGTst	295	CCCAAuaAAcu CCAGGccuTsT	296	AD-15143				16		
841-859	CUCCUA-GACACCAC-CAUACTsT	297	GUAUGCUG-GUGUCUAG-GAGTsT	298	AD-9521		50				
841-859	cuccuAGAcAc-cAGcAuAcTsT	299	GuAUGCUGGU-GUCuAG-GAGTsT	300	AD-9647		62				
842-860	UCCUACACAC-CAGCAUA-CATsT	301	UGUAUGCUG-GUGUCUAG-GATsT	302	AD-9611		48				
842-860	uccuAGAcAc-cAGcAuAcATsT	303	UGuAUGCUG-GUGUCuAG-GATsT	304	AD-9737		68				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
843-861	CCUAGACAC-CAG-CAUACAGTsT	305	CU-GUAUGCUG-GUGUCUAG-GTsT	306	AD-9592	46	55				
843-861	ccuAGAcAc-cAG-cAuAcAGTsT	307	CUGuAUGCUG-GUGUCuAG-GTsT	308	AD-9718		78				
847-865	GACACCAG-CAUACA-GAGUGTsT	309	CACUCU-GUAUGCUG-GUGUCTsT	310	AD-9561		64				
847-865	GAcAccAG-cAuAcA-GAGuGTsT	311	cACUCU-GuAUCCUU-GUCUCTsT	312	AD-9687		94				
855-873	CAUACAGAGU-GACCACCG-GTsT	313	CCGGUGGU-CACUCU-GUAUGTsT	314	AD-9636		42	41		2,10	
855-873	cAuAcAGAGu-GAccAccGGTsT	315	CCGGUGGU-cACUCU-GuAUGTsT	316	AD-9762		9	28		0,40	
860-878	AGAGUGAC-CACCG-GGAAAUTsT	317	AUUUCCCG-GUGGUCACU-CUTsT	318	AD-9540		45				
860-878	AGAGuGAccAc-cGGGAAAuTsT	319	AUUUCCCU-GUGGUcACU-CUTsT	320	AD-9666		81				
861-879	GAGUGACCAC-CG-GGAAAUCTsT	321	GAUUUC-CCUGGU-CAACUCTsT	322	AD-9535	48	73				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
861-879	GAUuGAccAc-cG-GGAAAucTsT	323	GAUUUCCCG-GUGGU-cACUCTsT	324	AD-9661		83				
863-881	GUGACCAC-CGGGAAA UCGATsT	325	UCUAUUUC-CCGGUGGUG-GUCACTsT	326	AD-9559		35				
863-881	GuGAccAccG-GUAAAuc-GATsT	327	UCGAUUUC-CCGGUGGU-cACTsT	328	AD-9685		77				
865-883	GACCACCG-GGAAAUCGAG-GTsT	329	CCUCUAUUUC-CCUCU-UCUCTsT	330	AD-9533		100				
865-883	GAccAccG-GGAAAucGAG-GTsT	331	CCUCGAUUUC-CCGGUG-GUCTsT	332	AD-9659		88				
866.884	ACCACCG-GGAAAUCGAG-GGTsT	333	CCCUCGAUU-UCCCGGUG-GUTsT	334	AD-9612		122				
866-884	AccAc-cGCCAAAucGA-UUCTsT	335	CCCUCGAUU-UCCCGGUG-GUTsT	336	AD-9738		83				
867-885	CCAC-CCCUAAAUC-CGACCGCTsT	337	GCCCUCCAUU-UCCCG-GUCGTsT	338	AD-9557	75	96				
807-885	ccAccUG-GAAAucGAG-GGcTsT	339	GCCCUCGAU-UUCCCCCUG-GTsT	340	AD-9683		48				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
875-893	AAAUCGAG-GGCAGGGU-CAUTsT	341	AUGACCCUC-CCCUCGAUU-UTsT	342	AD-9531		31	32		0,53	
875-893	AAAucGACGU-cAGGGucAuTsT	343	AUGAC-CCUGCCCUC-GAUUUTsT	344	AD-9657		23	29		0,66	
875-893	AfaAfuCfgAfgGf-gCfaGfgGfuC-faUfTTsT	345	p-aUfgAfcCfcUf-gCfcCfuCfgAfu-UfuTsT	346	AD-14673				81		
875-893	AAAUfCfGAG-GUCfAUGGUfC-fAUfTsT	347	AUfGACfCfCUf-GCfCfCUfCf-GAUfUfTs	348	AD-14683				56		
875-893	AaAuCgAg-GgCaGgGu-CaUTsT	349	p-aUfgfgAfcCf-cUfgCfcGfuCf-gAfuUfuTsT	350	AD-14693				56		
875-893	AaAuCgAg-GgCaGgGu-CaUTsT	351	AUfGACfCfCfUf-GCfCfUfCfGAU-fUfUfTsT	352	AD-14703				68		
875-893	AfaAfuCfgAfgGf-gCfaGfgCfuC-faUfTsT	353	AUGACccUC-ccCUCCAuu-uTsT	354	AD-14713				55		
875-893	AAAUfCfGAG-GGCfACCGUfC-fAUtTsT	355	AUGAC-ccUGccCUCGA-uuuTsT	356	AD-14723				24		
875-893	AaAuCgAg-GgCaGgGu-CaUTsT	357	AUCACccUUc-cCUCCAuuuTsT	358	AD-14733				34		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
875-893	CfgGfcAfcCf-cUfcAfuAfgGfc-CfuCfTsT	359	p-cAfgGfeC-fuAfuGfaGf-gCfuGfcCfgTsT	360	AD-15079				85		
875-893	CfGGCfACfCfC-fUfCfAUfAG-GCfCfUfUfGTsT	361	CfAGGCfCfU-fAUfGAGGGUf-GCfCfGTsT	362	AD-15089				54		
875-893	CgGcAcCcU-cAuAgGcCu-UTsT	363	p-cAfgGfc-CfuAfuGfaUfg-GfuGfcCfgTsT	364	AD-15099				70		
875-893	CgGcAcCcU-cAuAgCcCuCTsT	365	CfACGCfCfU-fAUfGAGGGUf-GCfCfGTsT	366	AD-15109				67		
875-893	CfgCfcAfcCf-cUfcAfuAfgGfc-CfuGfTsT	367	CACGCcuAU-gaGGGUC-ccgTsT	368	AD-15119				67		
875-893	CfGGCfACfTC-fUfCfAUfAG-GCfCfUfGTsT	369	CAGGCcuAU-gaG-GGUGccgTsT	370	AD-15129				57		
875-893	CgCcAcCcU-cAuAg-GcCuCTsT	371	CAGGCcuAU-gaG-GGUGccgTsT	372	AD-15139				69		
877-895	AUCGAGCG-CAGGGU-CAUCGTsT	373	CCAUGAC-CCUGCCCUCG UTsT	374	AD-9542		160				
877-895	AucGAG-GGcAGGGu-cAuGGTsT	375	CcAUAC-CCUGCCCUC-GAUTsT	376	AD-9668		92				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
878-896	cCAGGcAG-GGucAuG-GucTsT	377	GACcAU-GACUGCCUGC CCUCGTsT	378	AD-9739		109				
880-898	GAGGGCAG-GGUCAUGGU-CATsT	379	UCACCAUUAC-CCUGCCCUCT sT	380	AD-9637	56	83				
880-898	GAGGGcAG-GGucAuGGuc-ATsT	381	UCACcAUGAC-CCUGCCCUCT sT	382	AD-9763		79				
882-900	GGGCAGGGU-CAUGGUCAC-CTsT	383	GGUCACCAU-GAC-CCUGCCCTsT	384	AD-9630		82				
882-900	GGGcAGGGu-cAuGGucAc-cTsT	385	GGUGACcAU-GAC-CCUGCCCTsT	386	AD-9756		63				
885-903	CAGGGU-CAUGGUCAC-CGACTsT	387	GUCGGUGAC-CAUCAC-CCUCTsT	388	AD-9593		55				
885-903	cAGGGucAuG-GucAccGAcTsT	389	GUCCGUGAC-cAUUAC-CCUCTsT	390	AD-9719		115				
886-904	AGCGUCAUC-CUCAC-CGACUTsT	391	ACUCCGUGAC-CAUGAC-CCUTsT	392	AD-9601		111				
886-904	AGGGucAuG-GucAc-cGAcuTsT	393	AGUCGGU-CACcAUCAC-CCUTsT	394	AD-9727		118				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
892-910	AUGGUCAC-CGACUUCG-GATsT	395	UCUC-GAAGUCGGU-GACCAUTsT	396	AD-9573		36	42		1,60	
892-910	AuGGucAc-cGAcuucGA-GATsT	397	UCUC-GAAGUCGGU-GACcAUTsT	398	AD-9699		32	36		2,50	
899-917	CCGACUUCGA-GAAU-GUGCCTT	399	GGCACAU-UCUC-GAAGUCGGTT	400	AD-15228				26		
921-939	GGAGGACG-GGACCCGCU-UCTT	401	GAAGCG-GGUCCCGUC-CUCCTT	402	AD-15395				53		
993-1011	CACCG-GCCGUGAUC-CCCCCTsT	403	GCCGGCAUC-CCUGCCGCUG TsT	404	AD-9602		126				
993-1011	cAGcGGcuG-GGAuGccG-GcTsT	405	GCCGGcAUC-CCG-GCCGCUGTsT	406	AD-9728		94				
1020-1038	GGGUGCCAG-CAUGCG-CAGCTT	407	GCUGCG-CAUGGCUG-GCACCCTT	408	AD-15386				45		
1038-1056	CCUGCGCGUG CU-CAACUGCTsT	409	GCAUUUGACi-CACGCG-CACiGTsT	410	AD-9580		112				
1038-1056	ccuGcGcGuGcu cAAcuGcTsT	411	GcAGUUGAG-cACGCGcAG-GTsT	412	AD-9706		86				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1040-1058	UGCCCGUGCU CAACUGCCATs T	413	UCGCAGUU-GAGCACGCG-CATsT	414	AD-9581		35				
1040-1058	uGcCcUuGcu-cAAcuCccATsT	415	UGGcAGUU-GAGcACGCG-cATsT	416	AD-9707		81				
1042-1060	CGCGUGCU-CAACUUC-CAAGTsT	417	CUUGGCAGU-UGAGCAC-CCGTsT	418	AD-9543		51				
1042-1060	cGcGuGcuAAcu GccAAGTsT	419	CUUGGcAGUU-GAG-cACGCGTsT	420	AD-9669		97				
1053-1071	CUGCCAAG-GGAAG-GGCACGTsT	421	CGUGCCCU-UCCCUUG-GCAGTsT	422	AD-9574		74				
1053-1071	cuGccAAG-GGAAG-GGcAcGTsT	423	CCUCCCCUUC-CCUUC-CcAGTsT	424	AD-9700						
1057-1075	CAAGGCAACG-GCACGGU-UATT	425	UAAC-CGUGCCCU-UCCCUUGTT	426	AD-15320				26		
1058-1076	AAGGGAAG-GGCACGGU-UAGTT	427	CUAACCGUUC-CCUUCCCU-UTT	428	AD-15321				34		
1059-1077	AGGGAAGCG-CACGCU-UAGCTT	429	GCUAAC-CGUGCCCU-UCCCUTT	430	AD-15199				64		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1060-1078	GGGAAG-GGCACGGU-UACCGTT	431	CGCUAAC-CGUGCCCU-UCCCTT	432	AD-15167				86		
1061-1079	GGAAG-GGCACGGU-UAGCGGTT	433	CCGCUAAC-CGUGCCCU-UCCTT	434	AD-15164				41		
1062-1080	GAAGGGCAG-CACGGU-UAGCGGCTT	435	CCCCCUAAC-CCUCCCCU-UCTT	436	AD-15166				43		
1063-1081	AAGGGCACG-GUUAGCG-GCATT	437	UGCCGCUAAC-CGUGCCCU-UTT	438	AD-15322				64		
1064-1082	AG-GGCACGCU-UAGCG-GCACTT	439	GUGCCCGCUAAC-CGUGCCCUTT	440	AD-15200				46		
1068-1086	CACGGU-UAGCGGCAC-CCUCTT	441	GAG-GGUGCCGCUAACCGUGTT	442	AD-15213				27		
1069-1087	ACGGU-UAGCGGCAC-CCUCATT	443	UGAGGGUC-CCGCUAAC-CCUTT	444	AD-15229				44		
1072-1090	GUUAGCUG-CACCCU-CAUAGTT	445	CUAUGAG-GGUGCCGCUAACTT	446	AD-15215				49		
1073-1091	UUAGCG-GCACCCU-CAUAGGTT	447	CCUAUGAUG-GUGCCGCUAATT	448	AD-15214				101		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1076-1094	GCGGCAC-CCU-CAUAUGCCUTsT	449	ACCCCUAU-CACGOUGCCCCTsT	450	AD-9315		15	32		0,98	
1079-1097	GCACCCU-CAUAG-GCCUGGATsT	451	UCCACGCCUAUGAG-GGUGCTsT	452	AD-9326		35	51			
1085-1103	UCAUAC-CCCUGCAUUU-UAUTsT	453	AUAAACUC-CACCCCUAU-GATsT	454	AD-9318		14	37		0,40	
1090-1108	GGCCUCGAG-UUUAUUCG-CATsT	455	UCCCAAUAAACUCCAG-GCCTsT	456	AD-9323		14	33			
1091-1109	GCCUGGAGU-UUAUUC-CGAATsT	457	UUC-CCAAUAAACUCCAGGCTsT	458	AD-9314		11	22		0.04	
1091-1109	GccuCGAGuu-uAuucCGAATsT	459	UUC-CGAAuAAACUCcAGGCTsT	460	AD-10792					0,10	0,10
1091-1109	GccuGGAGuu-uAuucGGAATsT	461	UUC-CGAAUAACUC-CAGGCTsT	462	AD-10796					0,1	0,1
1093-1111	CUGGAGUU-UAUUCG-GAAAATsT	463	UUUUC-CGAAUAAACUCCAGTs	464	AD-9638		101				
1093-1111	cuGGAGuuuAu-ucGGAAAATsT	465	UUUUC-CGAAuAAAACUCcAGTsT	466	AD-9764		112				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1095-1113	GGAGUUUAU-UCCGAAAAGC TsT	467	GCUUUUC-CGAAUAAACU CCTsT	468	AD-9525		53				
1095-1113	GGAGuuuAu-ucG-GAAAAGcTsT	469	GCUUUUC-CGAAuAAACUC CTsT	470	AD-9651		58				
1096-1114	GAUUUUAUUC-CUAAAAUC-CTsT	471	GGCUUUUC-CGAAUAAACU CTsT	472	AD-9560		97				
1096-1114	GAGuuuAuucG-GAAAAUccTsT	473	GGCUUUUC-CGAAuAAACUC TsT	474	AD-9686		111				
1100-1118	UUAUUCG-CAAAACCCAC-CUTsT	475	AGCUGGCUU-UUC-CCAAUAATsT	476	AD-9536		157				
1100-1118	uuAuucG-CAAAAGccAC-cuTsT	477	AGCUUUCUUU-UCCGAAuAATs T	478	AD-9662		81				
1154-1172	CCCUGGCG-GUG-GGUACAGTsT	479	CUGUACCCAC-CCGCCAG-GGTsT	480	AD-9584	52	68				
1154-1172	cccuGGcG-GGuG-GGuAcAGTsT	481	CUGuACCcAC-CCGCcAG-GGTsT	482	AD-9710		111				
1155-1173	CCUGGCG-GGUACAGCTT	483	GCUGUAC-CCAC-CCGCCAGGTT	484	AD-15323				62		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1157-1175	UGGCGGGUG-GGUACAGCCG TsT	485	CGGCUGUAC-CCAC-CCGCCATsT	486	AD-9551		91				
1157-1175	uGGcGGGuG-GGuAcAGccGT sT	487	CGGCCUCuAC-CeAC-CCGCcATsT	488	AD-9677		62				
1158-1176	CCCGGGUG-GCUACAGCCC CTT	489	GCGGCU-GUACCCAC-CCGCCTT	490	AD-15230				52		
1162-1180	CCUGCCUACA CCCCCGUC-CTT	491	GGACGCGCGC UGUACCCAC-CTT	492	AD-15231				25		
1164-1182	UG-GGUACAGCCG CGUCCUCTT	493	GAGGACGCG-GCUGUAC-CCATT	494	AD-15285				36		
1172-1190	CCCGCCUC-CUCAAC-CCCCCTT	495	GCGGCGUU-GAGGACGCG-GCTT	496	AD-15396				27		
1173-1191	CCGCCUCCU-CAACGCCGCC TT	497	GGCCGCCUU-GAGCACGCG-GTT	498	AD-15397				56		
1216-1234	GUCGUGCUG-GUCAC-CGCUGTsT	499	CAGCGGU-GACCAGCAC-GACTsT	500	AD-9600		112				
1216-1234	GucGuGcuGGu-cAccGcuGTsT	501	cAGCGGUGAC-cAGcAC-GACTsT	502	AD-9726		95				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1217-1235	UCGUGCUG-GUCACCU-CUGCTs	503	GCAGCGGU-GACCAGCAC-GATsT	504	AD-9606		107				
1217-1235	ucGuGcuGGu-cAccGcuGcTsT	505	GcAGCGUGAC-cAGcACGATsT	506	AD-9732		105				
1223-1241	UGGUCAC-CGCUGCCG-GCAATsT	507	UUGCCG-GCAGCGGU-GACCATsT	508	AD-9633	56	75				
1223-1241	uGGucAc-cGcuGccG-GcAATsT	509	UUGCCG-GcAGCGGU-GACcATsT	510	AD-9759		111				
1224-1242	GGUCAC-CGCUGCCG-GCAACTsT	511	GUUGCCG-GCAGCGGU-GACCTsT	512	AD-9588		66				
1224-1242	GGucAc-cGcuGccG-GcAAcTsT	513	GUUGCCG-GcAGCGGU-GACCTsT	514	AD-9714		106				
1227-1245	CAC-CGCUGCCG-GCAACUUCTsT	515	GAAGU-UGCCG-GCAGCG-GUGTsT	516	AD-9589	67	85				
1227-1245	cAccGcuGccG-GcAAcuucTsT	517	GAAGU-UGCCG-GcAGCG-GUGTsT	518	AD-9715		113				
1229-1247	CCGCUGCCG-GCAACUUC-CGTsT	519	CGGAAGU-UGCCG-GCAGCGGTsT	520	AD-9575		120				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1229-1247	ccGcuGccG-GcAAcuuc-cGTsT	521	CGGAAGU-UGCCG-GcAGCGGTsT	522	AD-9701		100				
1230-1248	CGCUGCCG-GCAACUUCCG-GTsT	523	CCGGAAGU-UGCCG-GCAGCGTsT	524	AD-9563		103				
1230-1248	cGcuGccG-GcAAcuuccG-GTsT	525	CCGGAAGU-UGCCG-GcAGCGTsT	526	AD-9689		81				
1231-1249	GCUGCCG-GCAACUUCCG-GGTsT	527	CCCGGAAGU-UGCCG-GCAGCTsT	528	AD-9594	80	95				
1231-1249	GcuGccG-GcAAcuuccG-GGTsT	529	CCCGGAAGU-UGCCG-GcAGCTsT	530	AD-9720		92				
1236-1254	CGGCAACUUC-CGGGAC-GAUTsT	531	AUCGUCCCG-GAAGU-UGCCGTsT	532	AD-9585		83				
1236-1254	cGGcAAcuuc-cGGGAc-GAuTsT	533	AUCGUCCCG-GAAGU-UGCCGTsT	534	AD-9711		122				
1237-1255	GGCAACUUC-CGGGAC-GAUGTsT	535	CAUCGUC-CCGGAAGU-UGCCTsT	536	AD-9614		100				
1237-1255	GGcAAcuuccG-GGAcG AuGTsT	537	cAUCGUCCCG-GAAGU-UGCCTsT	538	AD-9740		198				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1243-1261	UUCCGGAC-GAUGCCUGCC TsT	539	GGCAG-GCAUCGUC-CCGGAATsT	540	AD-9615		116				
1243-1261	uuccGGGAc-GAuGccuGccTs T	541	GGcAG-GcAUCGUC-CCGGAATsT	542	AD-9741		130				
1248-1266	GGAC-GAUGCCUGCC UCUACTsT	543	GUAGAG-GCAG-GCAUCGUC-CTsT	544	AD-9534		32	30			
1248-1266	GGAC-GAUGCCUGCC UCUACTsT	545	GUAGAG-GCAG-GCAUCGUC-CTsT	546	AD-9534		32				
1248-1266	GGAc-GAuGccuGccu-cuAcTsT	547	GuAGAGGcAG-GcAUCGUC-CTsT	548	AD-9660		89	79			
1279-1297	GCUCCCGAG-GUCAU-CACAGTT	549	CUGUGAU-GACCUCG-GGAGCTT	550	AD-15324				46		
1280-1298	CUCCCGAG-GUCAU-CACAGUTT	551	ACUGUGAU-GACCUCG-GGAGTT	552	AD-15232				19		
1281-1299	UCCCGAGGU-CAUCACAGU-UTT	553	AACUGUGAU-GACCUCG-GGATT	554	AD-15233				25		
1314-1332	CCAAGAC-CAGCCGGU-GACCTT	555	GGUCACCG-GCUGGUCU-UGGTT	556	AD-15234				59		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1315-1333	CAAGAC-CAGCCGGU-GACCCTT	557	GGGUCACCG-GCUGGUCU-UGTT	558	AD-15286				109		
1348-1366	ACCAACUUUG-GCCGCU-GUGTsT	559	CACAGCG-GCCAAAGUUG-GUTsT	560	AD-9590		122				
1348-1366	AccAAcuuuG-GccGcuGuGTsT	561	cAcAGCG-GCcAAAGUUG-GUTsT	562	AD-9716		114				
1350-1368	CAACUUUG-GCCGCUGU-GUGTsT	563	CACACAGCG-GCCAAAGU-UGTsT	564	AD-9632		34				
1350-1368	cAAcuuuG-GccGcuGu-GuGTsT	565	cAcAcAGCG-GCcAAAGU-UGTsT	566	AD-9758		96				
1360-1378	CGCUGUGUG-GACCUCUU-UGTsT	567	CAAAGAGGUC-CACACAGCGTsT	568	AD-9567		41				
1360-1378	cGcuGuGuG-GAccucuu-uGTsT	569	cAAAGAGGUC-cAcAcAGCGTsT	570	AD-9693		50				
1390-1408	GACAUCAUUG-GUGCCUC-CATsT	571	UGGAGGCAC-CAAUGAU-GUCTsT	572	AD-9586	81	104				
1390-1408	GAcAucAuuG-GuGccuccATsT	573	UGGAGGcAC-cAAUGAU-GUCTsT	574	AD-9712		107				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1394-1412	UCAUUG-GUGCCUC-CAGCGATsT	575	UCGCUGGAG-GCACCAAU-GATsT	576	AD-9564		120				
1394-1412	ucAuuG-GuGccuc-cAGcGATsT	577	UCGCUGGAG-GcACcAAU-GATsT	578	AD-9690		92				
1417-1435	AGCACCUGCU-UUGUGU-CACTsT	579	GU-GACACAAAG-CAG-GUGCUTsT	580	AD-9616	74	84				
1417-1435	AGcAccuGcuuu-GuGucAcTsT	581	GU-GAcAcAAAG-cAGGUGCUTsT	582	AD-9742		127				
1433-1451	CACAGAGUG-GGACAUCA-CATT	583	UGUGAUGUC-CCACUCU-GUGTT	584	AD-15398				24		
1486-1504	AUGCU-GUCUGCCGAG CCGGTsT	585	CCGGCUCG-GCAGACAG-CAUTsT	586	AD-9617		111				
1486-1504	AuGcu-GucuGccGAGcc GGTsT	587	CCGGCUCG-GcAGAcAG-cAUTsT	588	AD-9743		104				
1491-1509	GUCUGCCGAG CCG-GAGCUCTsT	589	GAGCUCCG-GCUCGGCA-GACTsT	590	AD-9635	73	90				
1491-1509	GucuGccGAGcc GGAGcucTsT	591	GAGCUCCG-GCUCGGcA-GACTsT	592	AD-9761		83				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1521-1539	GUUGAGGCA-GAGACU-GAUCTsT	593	GAUCAGUCU-CUGCCU-CAACTsT	594	AD-9568		76				
1521-1539	GuuGAGGcA-GAGAcu-GAucTsT	595	GAUcAGUCU-CUGCCU-cAACTsT	596	AD-9694		52				
1527-1545	GCAGAGACU-GAUCCACU-UCTsT	597	GAAGUGGAU-CAGUCU-CUGCTsT	598	AD-9576		47				
1527-1545	GcAGAGAcu-GAuccAcuucTsT	599	GAAGUGGAU-cAGUCU-CUGCTsT	600	AD-9702		79				
1529-1547	AGAGACU-GAUCCACU-UCUCTsT	601	GAGAAGUG-GAUCAGUCU-CUTsT	602	AD-9627		69				
1529-1547	AGAGAcuGAuc-cAcuucucTsT	603	GAGAAGUG-GAUcAGUCU-CUTsT	604	AD-9753		127				
1543-1561	UUCU-CUGCCAAA-GAUGUCATsT	605	UGACAUCUU-UGGCAGA-GAATsT	606	AD-9628		141				
1543-1561	uucucuGccAAA-GAuGucATsT	607	UGAcAUCUU-UGGcAGA-GAATsT	608	AD-9754		89				
1545-1563	CUCUGCCAAA-GAUGU-CAUCTsT	609	GAUGACAUCU-UUGGGCA-GAGTsT	610	AD-9631		80				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1545-1563	cucuGccAAA-GAuGucAucTsT	611	GAUGAcAUCU-UUGGcA-GAGTsT	612	AD-9757		78				
1580-1598	CUGAGGAC-CAGCG-GGUACUTsT	613	AGUAC-CCGCUGGUC-CUCAGTsT	614	AD-9595		31	32			
1580-1598	cuGAGGAc-cAGcG-GGAcuTsT	615	AGuAC-CCGCUGGUC-CUcAGTsT	616	AD-9721		87	70			
1581-1599	UGAGGAC-CAGCG-GGUACUGTsT	617	CAGUAC-CCGCUGG UCCUCATsT	618	AD-9544		68				
1581-1599	uGACGAc-cAGcG-GGuAcuGTsT	619	cAGuAC-CCGCUGGUC-CUcATsT	620	AD-9670		67				
1666-1684	ACUGUAUG-GUCAG-CACACUTT	621	AGUGUGCU-GAC-CAUACAGUTT	622	AD-15235				25		
1668-1686	UGUAUGGU-CAG-CACACUCGTT	623	CGAGU-GUGCUGAC-CAUACATT	624	AD-15236				73		
1669-1687	GUAUGGU-CAG-CACACUCG-GTT	625	CCGAGU-GUGCUGAC-CAUACTT	626	AD-15168				100		
1697-1715	GGAUG-GCCACAGCCG UGCTT	627	GCGACGGCU-GUGGCCAUC-CTT	628	AD-15174				92		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1698-1716	GAUG-GCCACAGCCG UCGCCTT	629	GGCGACG-GCUGUG-GCCAUCTT	630	AD-15325				81		
1806-1824	CAAGCUG-GUCUGCCG-GGCCTT	631	GGCCCGGCA-GACCAGCU-UGTT	632	AD-15326				65		
1815-1833	CUGCCG-GGCCCACAAC GCUTsT	633	AGCGUUGUG-GGCCCG-GCAGTsT	634	AD-9570	35	42				
1815-1833	cuGccG-GGcccAcAAcGc uTsT	635	AGCGUUGUG-GGCCCG-GcAGTsT	636	AD-9696		77				
1816-1834	UGCCG-GGCCCACAAC GCUUTsT	637	AAGCGUU-GUGGGCCCG-GCATsT	638	AD-9566		38				
1816-1834	uGccG-GGcccAcAAcGc uuTsT	639	AAGCGUU-GUGGGCCCG-GcATsT	640	AD-9692		78				
1818-1836	CCG-GGCCCACAAC GCUUUUTsT	641	AAAAGCGUU-GUGGGCCCG-GTsT	642	AD-9532		100				
1818-1836	ccG-GGcccAcAAcGc uuuuTsT	643	AAAAGCGUU-GUGGGCCCG-GTsT	644	AD-9658		102				
1820-1838	GGGCCCACAA CGCUUUUG-GTsT	645	CCAAAAGCGU-UGUG-GGCCCTsT	646	AD-9549		50				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
1820-1838	GGGcccAcAAc GcuuuuGGTsT	647	CcAAAAGCGU-UGUG-GGCCCTsT	648	AD-9675		78				
1840-1858	GGUGAGGGU-GUCUACGCCA TsT	649	UGGCGUA-GACACCCU-CACCTsT	650	AD-9541		43				
1840-1858	GGuGAGGGu-GucuAcGccATs T	651	UGGCGuA-GAcACCCU-cACCTsT	652	AD-9667		73				
1843-1861	GAGGGU-GUCUACGCCA-UUGTsT	653	CAAUGGCGUA-GACAC-CCUCTsT	654	AD-9550		36				
1843-1861	GAGGGu-GucuAcGccAu-uGTsT	655	cAAUCGCGuA-GAcAC-CCUCTsT	656	AD-9676		100				
1861-1879	GCCAG-GUGCUGCCUG CUACTsT	657	GUAGCAG-GCAGCAC-CUGGCTsT	658	AD-9571		27	32			
1861-1879	GccAG-GuGcuGccuGcu AcTsT	659	GuAGcAG-GcAGcACCUG-GCTsT	660	AD-9697		74	89			
1862-1880	CCAG-GUGCUGCCUG CUACCTsT	661	GGUAGCAG-GCAGCAC-CUGGTsT	662	AD-9572	47	53				
1862-1880	ccAG-GuGcuGccuGcu AccTsT	663	GGuAGcAG-GcAGcACCUG-GTsT	664	AD-9698		73				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2008-2026	AC-CCACAAGCCG CCUGUGCTT	665	GCACAGGCG-GCUUGUG-GGUTT	666	AD-15327				82		
2023-2041	GUGCUGAG-GCCACGAG-GUCTsT	667	GACCUCGUG-GCCUCAG-CACTsT	668	AD-9639		30	35			
2023-2041	GuGcuGAG-GccAcGAG-GucTsT	669	GACCUCGUG-GCCUcAG-cACTsT	670	AD-9765		82	74			
2024-2042	UGCUGAG-GCCACGAG-GUCATsT	671	UGAC-CUCGUG-GCCUCAG-CATsT	672	AD-9518		31	35		0,60	
2024-2042	UGCUGAG-GCCACGAG-GUCATsT	673	UGAC-CUCGUG-GCCUCAG-CATsT	674	AD-9518		31				
2024-2042	uGcuGAG-GccAcGAGGuc-ATsT	675	UGAC-CUCGUG-GCCUcAG-cATsT	676	AD-9644		35	37		2,60	
2024-2042	UfgCfuGfaGf-gCfcAfcGfaGf-gUfcAfTsT	677	p-uGfaCf-cUfcGfuGfgCf-cUfcAfgCfaTsT	678	AD-14672				26		
2024-2042	UfGCfUfGAG-GCfCfACfGAG-GUfCfATsT	679	UfGACfCfCUfCf-GUfGGCfC-fUfCfAGCfATsT	680	AD-14682				27		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2024-2042	UgCuGaG-gCcAcGaGgUc-ATsT	681	p-uGfaCf-cUfcGfuGfgCf-cUfcAfgCfaTsT	682	AD-14692				22		
2024-2042	UgCuGaG-gCcAcGaGgUc-ATsT	683	UfGACfCfUfCf-GUfGGCfC-fUfCfAGCfATsT	684	AD-14702				19		
2024-2042	UfgCfuGfaGf-gCfcAfcGfaGf-gUfcAfTsT	685	UGACCucGUg-gCCUCAgcaTsT	686	AD-14712				25		
2024-2042	UfGCfUfGAG-GCfCfACfGAG-GUfCfATsT	687	UGACCucGUg-gCCUCAgcaTsT	688	AD-14722				18		
2024-2042	UgCuGaG-gCcAcGaGgUc-ATsT	689	UGACCucGUg-gCCUCAgcaTsT	690	AD-14732				32		
2024-2042	GfuGfgUfcAfgCf-gGfcCfgGf-gAfuGfTsT	691	p-cAfuCfcCfg-GfcCfgCfuG-faCfcAfcTsT	692	AD-15078				86		
2024-2042	GUfGGUfCf-AGCfGGCfCfG-GGAUfGTsT	693	CfAUfCfCfCfG-GCfCfGCfUf-GACfCfACfTsT	694	AD-15088				97		
2024-2042	GuGgUcAgCg-GcCg-GgAuGTsT	695	p-cAfuCfcCfg-GfcCfgCfuG-faCfcAfcTsT	696	AD-15098				74		
2024-2042	GuCgUcAgCg-GcCg-GgAuGTsT	697	CfAUfCfCfCfG-GCfCfGCfUf-GACfCfACfTsT	698	AD-15108				67		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2024-2042	GfuGfgUfcAfgCf-gGfcCfgGf-gAfuGfTsT	699	CAUCCcg-GCcgCUGAC-cacTsT	700	AD-15118				76		
2024-2042	GUfGGUfCf-AGCfGGCfCfG-GGAUfGTsT	701	CAUCCcg-GCcgCUGAC-cacTsT	702	AD-15128				86		
2024-2042	GuGgUcAgCg-GcCg-GgAuGTsT	703	CAUCCcg-GCcgCUGAC-cacTsT	704	AD-15138				74		
2030-2048	GGCCACGAG-GU-CAGCCCAATT	705	UUGGGCU-GACCUCGUG-GCCTT	706	AD-15237				30		
2035-2053	CGAGGU-CAGCCCAAC-CAGUTT	707	ACUGGUUG-GGCUGAC-CUCGTT	708	AD-15287				30		
2039-2057	GU-CAGCCCAAC-CAGUGCGUTT	709	ACGCACUGG-UUGGGCU-GACTT	710	AD-15238				36		
2041-2059	CAGCCCAAC-CAGUGCGUG-GTT	711	CCACGCACUG-GUUG-GGCUGTT	712	AD-15328				35		
2062-2080	CACAGGGAG-GCCAGCAUC-CTT	713	GGAUGCUG-GCCUCCCU-GUGTT	714	AD-15399				47		
2072-2090	CCAGCAUC-CACGCUUC-CUGTsT	715	CAG-GAAGCGUG-GAUGCUG-GTsT	716	AD-9582		37				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2072-2090	ccAGcAuc-cAcGcuuc-cuGTsT	717	cAG-GAAGCGUG-GAUGCUG-GTsT	718	AD-9708		81				
2118-2136	AGUCAAGGAG-CAUG-GAAUCTsT	719	GAUUC-CAUGCUCCUU-GACUTsT	720	AD-9545		31	43			
2118-2136	AGucAAGGAG-cAuGGAAucTsT	721	GAUUC-cAUGCUCCUU-GACUTsT	722	AD-9671		15	33		2,50	
2118-2136	AfgUfcAfaGfgAf-gCfaUfgG-faAfuCfTsT	723	p-gAfuUfcCfaUf-gCfuCfcUfuG-faCfuTsT	724	AD-14674				16		
2118-2136	AGUfCAAG-GAGCfAUfG-GAAUfCfTsT	725	GAUfUfCfCfAUf-GCfUfCfUfUf-GACfUfTs T	726	AD-14684				26		
2118-2136	AgUcAaGgAg-CaUgGaAuCTsT	727	p-gAfuUfcCfaUf-gCfuCfcUfuG-faCfuTsT	728	AD-14694				18		
2118-2136	AgUcAaGgAg-CaUgGaAuCTsT	729	GAUfUfCfCfAUf-GCfUfCfCfUfUf-GACfUfTs T	730	AD-14704				27		
2118-2136	AfgUfcAfaGfgAf-gCfaUfgG-faAfuCfTsT	731	GAUUC-caUGcuCCUU-GacuTsT	732	AD-14714				20		
2118-2136	AGUfCfAAG-GAGCfAUfG-GAAUfCfTsT	733	GAUUC-caUGcuCCUU-GacuTsT	734	AD-14724				18		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2118-2136	AgUcAaGgAg-CaUgGaAuCTsT	735	GAUUC-caUGcuCCUU-GacuTsT	736	AD-14734				18		
2118-2136	GfcGfgCfaCfc-CfuCfaUfaGf-gCfcUfTsT	737	p-aGfgCfcUfaUf-gAfgGfgUf-gCfcGfcTsT	738	AD-15080				29		
2118-2136	GCfG-GCfACfCfCfUfC-fAUfAGGCfC-fUfTsT	739	AGGCfCfUfAUf-GAGGGUf-GCfCfGCfTsT	740	AD-15090				23		
2118-2136	GcGgCaCcCu-CaUaGgCcUTsT	741	p-aGfgCfcUfaUf-gAfgGfgUf-gCfcGfcTsT	742	AD-15100				26		
2118-2136	GcGgCaCcCu-CaUaGgCcUTsT	743	AGGCfCfUfAUf-GAGGGUf-GCfCfGCfTsT	744	AD-15110				23		
2118-2136	GfcGfgCfaCfc-CfuCfaUfaGf-gCfcUfTsT	745	AGGCCuaU-Gag-GGUGCcgcTsT	746	AD-15120				20		
2118-2136	GCfG-GCfACfCfCfUfC-fAUfAGGCfC-fUfTsT	747	AGGCCuaU-Gag-GGUGCcgcTsT	748	AD-15130				20		
2118-2136	GcGgCaCcCu-CaUaGgCcUTsT	749	AGGCCuaU-Gag-GGUGCcgcTsT	750	AD-15140				19		
2122-2140	AAGGAGCAUG-GAAUCCCG-GTsT	751	CCGGGAUUC-CAUGCUCCU-UTsT	752	AD-9522		59				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2122-2140	AAGGAGcAuG-GAAucccGGTsT	753	CCGGGAUUC-cAUGCUCCU-UTsT	754	AD-9648		78				
2123-2141	AGGAGCAUG-GAAUCCCG-GCTsT	755	GCCGGGAU-UCCAUGCUC-CUTsT	756	AD-9552		80				
2123-2141	AGGAGcAuG-GAAucccG-GcTIsT	757	GCCGGGAU-UCcAUGCUC-CUTsT	758	AD-9678		76				
2125-2143	GAGCAUG-GAAUCCCG-GCCCTsT	759	GGGCCGGGA-UUC-CAUGCUCTsT	760	AD-9618		90				
2125-2143	GAGcAuG-GAAucccG-GcccTsT	761	GGGCCGGGA-UUC-cAUGCUCTsT	762	AD-9744		91				
2230-2248	GCCUACGCCG UAGACAACATT	763	UGUU-GUCUACG-GCGUAGGCTT	764	AD-15239				38		
2231-2249	CCUACGCCGU AGACAACACTT	765	GUGUU-GUCUACG-GCGUAGGTT	766	AD-15212				19		
2232-2250	CUACGCCGUA-GACAAC ACGTT	767	CGUGUU-GUCUACG-GCGUAGTT	768	AD-15240				43		
2233-2251	UACGCCGUA-GACAACACGU TT	769	ACGUGUU-GUCUACG-GCGUATT	770	AD-15177				59		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2235-2253	CGCCGUA-GACAACACGU-GUTT	771	ACACGUGUU-GUCUACG-GCGTT	772	AD-15179				13		
2236-2254	GCCGUA-GACAACACGU-GUGTT	773	CACACGUGUU-GUCUACG-GCTT	774	AD-15180				15		
2237-2255	CCGUA-GACAACACGU-GUGTT	775	ACACACGUGU-UGUCUACG-GTT	776	AD-15241				14		
2238-2256	CGUA-GACAACACGU-GUGUATT	777	UACACACGUG-UU-GUCUACGTT	778	AD-15268				42		
2240-2258	UA-GACAACACGU-GUGUAGUTT	779	AC-UACACACGUG-UUGUCUATT	780	AD-15242				21		
2241-2259	AGACAACACG UGU-GUAGUCTT	781	GAC-UACACACGUG-UUGUCUTT	782	AD-15216				28		
2242-2260	CACAACACGU-GUGUAGU-CATT	783	UGAC-UACACACGUG-UUGUCTT	784	AD-15176				35		
2243-2261	ACAACACCU-GUGUAGU-CAG_T^T	785	CUGAC-UACACACGUG-UUGUTT	786	AD-15181				35		
2244-2262	CAACACGUGU-GUAGUCAG-GTT	787	CCUGAC-UACACACGUG-UUGTT	788	AD-15243				22		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2247-2265	CACGUGU-GUAGUCAG-GAGCTT	789	GCUCCUGAC-UACACACGUG TT	790	AD-15182				42		
2248-226	ACGUGU-GUAGUCAG-GACCTT	791	GGCUCCU-GAC-UACACACGUT T	792	AD-15244				31		
2249-2267	CGUGU-GUAGUCAG-GAGCCGTT	793	CGGCUCCU-GAC-UACACACGTT	794	AD-15387				23		
2251-2269	UGUGUAGU-CAGGAGCCG-GGTT	795	CCCGGCUC-CUGACUACA-CATT	796	AD-15245				18		
2257-2275	GUCAG-GAGCCG-GGACGUCATsT	797	UGACGUC-CCGGCUCCU-GACTsT	798	AD-9555		34				
2257-2275	GucAG-GAGccG-GGAcGucATsT	799	UGACGUC-CCGGCUCCU-GACTsT	800	AD-9681		55				
2258-2276	UCAG-GAGCCG-GGACGU-CAGTsT	801	CUGACGUC-CCGGCUCCU-GATsT	802	AD-9619	42	61				
2258-2276	ucAGGAGccG-GGAcGu-cAGTsT	803	CUGACGUC-CCGGCUCCU-GATsT	804	AD-9745		56				
2259-2277	CAGGAGCCG-GGACGU-CAGCTsT	805	GCUGACGUC-CCGGCUC-CUGTsT	806	AD-9620	44	77				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2259-2277	cAGGAGccG-GGAcGu-cAGcTsT	807	GCUGACGUC-CCGGCUC-CUGTsT	808	AD-9746		89				
2263-2281	AGCCG-GGACGUCAG-CACUATT	809	UAGUGCU-GACGUCCCG-GCUTT	810	AD-15288				19		
2265-223	CCGGGACGU-CAGCACUA-CATT	811	UGUAGUGCU-GACGUCCCG-GTT	812	AD-15246				16		
2303-2321	CCGU-GACAGCCGU-UGCCAUTT	813	AUGGCAACG-GCUGUCACG-GTT	814	AD-15289				37		
2317-2335	GCCAUCUGCU GCCG-GAGCCTsT	815	GGCUCCG-GCAGCA-GAUGGCTsT	816	AD-9324		59	67			
2375-2393	CCCAUCCCAG-GAUGGGU-GUTT	817	ACACCCAUC-CUGGGAUG-GGTT	818	AD-15329				103		
2377-2395	CAUCCCAG-GAUGGGU-GUCUCUTT	819	AGACAC-CCAUCCUG-GGAUGTT	820	AD-15330				62		
2420-2438	AGCUU-UAAAAUGGU-UCCGATT	821	UCGGAACCAU-UU-UAAAGCUTT	822	AD-15169				22		
2421-2439	GCUU-UAAAAUGGU-UCCGACTT	823	GUCGGAACCA-UUU-UAAAGCTT	824	AD-15201				6		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2422-2440	CUUUAAAAUG-GUUC-CGACUTT	825	AGUCGGAAC-CAUUU-UAAAGTT	826	AD-15331				14		
2423-2441	UUUAAAAUGG-UUCCGACU-UTT	827	AAGUCGGAAC-CAUUUUAAATT	828	AD-15190				47		
2424-2442	UUAAAAUGGU-UCCGACU-UGTT	829	CAAGUCG-GAACCAUUU-UAATT	830	AD-15247				61		
2425-2443	UAAAAUGGU-UCCGACUU-GUTT	831	ACAAGUCG-GAACCAUUU-UATT	832	AD-15248				22		
2426-2444	AAAAUGGUUC-CGACUU-GUCTT	833	GACAAGUCG-GAACCAUUU-UTT	834	AD-15175				45		
2427-2445	AAAUGGUUC-CGACUUGUC-CTT	835	GGACAAGUCG GAACCAUU-UTT	836	AD-15249				51		
2428-2446	AAUGGUUC-CGACUUGUC-CCTT	837	GGGACAAGUC GGAACCAU-UTT	838	AD-15250				96		
2431-2449	GGUUCCGAC-UUGUCCCU-CUTT	839	AGAG-GGACAAGUCG GAACCTT	840	AD-15400				12		
2457-2475	CUCCAUG-GCCUGGCAC-GAGTT	841	CUCGUGCCAG GCCAUG-GAGTT	842	AD-15332				22		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2459-2477	CCAUG-GCCUGGCAC-GAGGGTT	843	CCCUCGUGCC AGGCAUGGTT	844	AD-15388				30		
2545-2563	GAACUCACU-CACUCUG-GGUTT	845	ACCCAGAGU-GAGUGAGU-UCTT	846	AD-15333				20		
2549-2567	UCACUCACU-CUG-GGUGCCUTT	847	AGGCACCCA-GAGUGAGU-GATT	848	AD-15334				96		
2616-2634	UUUCACCAUU-CAAACAG-GUTT	849	ACCUGUUU-GAAUGGU-GAAATT	850	AD-15335				75		
2622-2640	CAUU-CAAACAGGUC-GAGCUTT	851	AGCUCGACCU-GUUU-GAAUGTT	852	AD-15183				16		
2623-2641	AUUCAAACAG-GUC-GAGCUGTT	853	CAGCUCGAC-CUGUUU-GAAUTT	854	AD-15202				41		
2624-2642	UUCAAACAG-GUCGAGCU-GUTT	855	ACAGCUCGAC-CUGUUU-GAATT	856	AD-15203				39		
2625-2643	UCAAACAG-GUCGAGCU-GUGTT	857	CACAGCUC-GACCUGUUU-GATT	858	AD-15272				49		
2626-2644	CAAACAGGUC-GAGCU-GUGCTT	859	GCACAGCUC-GACCUGUU-UGTT	860	AD-15217				16		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2627-2645	AAACAGGUC-GAGCU-GUGCUTT	861	AGCACAGCUC-GACCUGUU-UTT	862	AD-15290				15		
2628-2646	AACAGGUC-GAGCU-GUGCUCTT	863	GAG-CACAGCUC-GACCUGUUTT	864	AD-15218				13		
2630-2648	CAGGUC-GAGCU-GUGCUCGGTT	865	CCGAG-CACAGCUC-GACCUGTT	866	AD-15389				13		
2631-2649	AGGUC-GAGCU-GUGCUCG-GGTT	867	CCCGAG-CACAGCUC-GACCUTT	868	AD-15336				40		
2633-2651	GUCGAGCU-GUGCUCG-GGUGTT	869	CACCCGAG-CACAGCUC-GACTT	870	AD-15337				19		
2634-2652	UCGAGCU-GUGCUCG-GGUGCTT	871	GCACCCGAG-CACAGCUC-GATT	872	AD-15191				33		
2657-2675	AGCUGCUC-CCAAU-GUGCCGTT	873	CGGCACAU-UGGGAG-CAGCUTT	874	AD-15390				25		
2658-2676	GCUGCUC-CCAAU-GUGCCGATT	875	UCGGCACAU-UGGGAG-CAGCTT	876	AD-15338				9		
2660-2678	UGCUCCCAAU-GUGCCGAUGTT	877	CAUCGGCACA-UUGGGAG-CATT	878	AD-15204				33		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2663-2681	UCCCAAU-GUGCCGAU-GUCCTT	879	GGACAUCG-GCACAUUG-GGATT	880	AD-15251				76		
2665-2683	CCAAU-GUGCCGAU-GUCCGUTT	881	ACGGACAUCG-GCACAUUG-GTT	882	AD-15205				14		
2666-2684	CAAU-GUGCCGAU-GUCCGUGTT	883	CACG-GACAUCG-GCACAUUGTT	884	AD-15171				16		
2667-2685	AAU-GUGCCGAU-GUCCGUGGTT	885	CCACG-GACAUCG-GCACAUUTT	886	AD-15252				58		
2673-2691	CCGAUGUC-CGUGGGCA-GAATT	887	UUCUGCCCAC GGACAUCG-GTT	888	AD-15339				20		
2675-2693	GAUGUC-CGUGGGCA-GAAUGTT	889	CAU-UCUGCCCACG GACAUCTT	890	AD-15253				15		
2678-2696	GUCCGUG-GGCAGAAU-GACUTT	891	AGUCAU-UCUGCCCACG GACTT	892	AD-15340				18		
2679-2697	UCCGUG-GGCAGAAU-GACUUTT	893	AAGUCAU-UCUGCCCACG GATT	894	AD-15291				17		
2683-2701	UGGGCA-GAAUGACUUU-UAUTT	895	AUAAAAGUCA-UUCUGCCCAT T	896	AD-15341				11		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2694-2712	ACUUUUAUU-GAGCUCUU-GUTT	897	ACAAGAGCU-CAAUAAAAGUTT	898	AD-15401				13		
2700-2718	AUUGAGCUCU-UGUUC-CGUGTT	899	CACGGAACAA-GAGCU-CAAUTT	900	AD-15342				30		
2704-2722	AGCUCUUGU-UCCGUGCCAG TT	901	CUGGCACG-GAACAA-GAGCUTT	902	AD-15343				21		
2705-2723	GCUCUUGU-UCCGUGCCAG GTT	903	CCUGGCACG-GAACAA-GAGCTT	904	AD-15292				16		
2710-2728	UGUUC-CGUGCCAG-GCAUUCTT	905	GAAUGCCUG-GCACGGAA-CATT	906	AD-15344				20		
2711-2729	GUUC-CGUGCCAG-GCAUUCATT	907	UGAAUGCCUG GCACG-GAACTT	908	AD-15254				18		
2712-2730	UUC-CGUGCCAG-GCAUUCAAATT	909	UU-GAAUGCCUG-GCACGGAATT	910	AD-15345				18		
2715-2733	CGUGCCAG-GCAUUCAAUC-CTT	911	GGAUU-GAAUGCCUG-GCACGTT	912	AD-15206				15		
2716-2734	GUGCCAG-GCAUUCAAUC-CUTT	913	AGGAUU-GAAUGCCUG-GCACTT	914	AD-15346				16		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2728-2746	CAAUCCUCAG-GUCUCCAC-CTT	915	GGUGGAGAC-CUGAGGAU-UGTT	916	AD-15347				62		
2743-2761	CACCAAGGAG-GCAGGAU-UCTsT	917	GAAUC-CUGCCUCCU-UGGUGTsT	918	AD-9577		33	31			
2743-2761	cAccAAGGAG-GcAGGAuucTsT	919	GAAUC-CUGCCUCCU-UGGUGTsT	920	AD-9703		17	26			
2743-2761	CfaCfcAfaGfgAf-gGfcAfgGfaU-fuCfTsT	921	p-gAfaUfc-CfuGfcCfuCf-cUfuGfgUfgTsT	922	AD-14678				22		
2743-2761	CfACfCfAAG-GAGGCfAG-GAUfUfCfTsT	923	GAAUfCfCfUf-GCfCfUfCfCfU-fUfGGUfGTs T	924	AD-14688				23		
2743-2761	CaCcAaGgAg-GcAgGaUuCTsT	925	p-gAfaUfc-CfuGfcCfuCf-cUfuGfgUfgTsT	926	AD-14698				23		
2743-2761	CaCcAaGgAg-GcAgGaUuCTsT	927	GAAUfCfCfUf-GCfCfUfCfCfU-fUfGGUfGTs T	928	AD-14708				14		
2743-2761	CfaCfcAfaGfgAf-gGfcAfgGfaU-fuCfTsT	929	GAAUC-cuGCcuCCU-UGgugTsT	930	AD-14718				31		
2743-2761	CfACfCfAAG-GAGGCfAG-GAUfUfCfTsT	931	GAAUC-cuGCcuCCU-UGgugTsT	932	AD-14728				25		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2743-2761	CaCcAaGgAg-GcAgGaUuCTsT	933	GAAUC-cuGCcuCCU-UGgugTsT	934	AD-14738				31		
2743-2761	GfgCfcUfgGfaG-fuUfuAfuUfcGf-gAfTsT	935	p-uCfcGfaAfuA-faAfcUfcCfaGf-gCfeTsT	936	AD-15084				19		
2743-2761	GGCfCfUfG-GAGUfUfUfAU-fUfCfGGATsT	937	UfCfCfGAAU-fAAACfUfCfCf-AGGCfCfTsT	938	AD-15094				11		
2743-2761	GgCcUgGaGuU-uAuUcGgATsT	939	p-uCfcGfaAfuA-faAfcUfcCfaGf-gCfcTsT	940	AD-15104				16		
2743-2761	GgCeUgGaGu-UuAuUcGgATsT	941	UfCfCfGAAU-fAAACfUfCfCf-AGGCfCfTsT	942	AD-15114				15		
2743-2761	GfgCfcUfgGfaG-fuUfuAfuUfeGf-gAfTsT	943	UCCGAauAAac UCCAGgccTsT	944	AD-15124				11		
2743-2761	GGCfCfUfG-GAGUfUfUfAU-fUfCfGGATsT	945	UCCGAauAAac UCCAGgccTsT	946	AD-15134				12		
2743-2761	GgCcUgGaGuU-uAuUcGgATsT	947	UCCGAauAAac UCCAGgccTsT	948	AD-15144				9		
2753-2771	GCAGGAUUC-UUCCCAUG-GATT	949	UCCAUGGGAA-GAAUC-CUGCTT	950	AD-15391				7		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2794-2812	UGCAG-GGACAAACAU CGUUTT	951	AACGAUGUUU-GUCCCUG-CATT	952	AD-15348				13		
2795-2813	GCAG-GGACAAACAU CGUUGTT	953	CAACGAUGUU-UGUC-CCUGCTT	954	AD-15349				8		
2797-2815	AG-GGACAAACAU CGUUGGGTT	955	CCCAACGAUG-UUUGUC-CCUTT	956	AD-15170				40		
2841-2859	CCCUCAUCUC-CAGCUAACUT T	957	AGUUAGCUG-GAGAUGAG-GGTT	958	AD-15350				14		
2845-2863	CAUCUC-CAGCUAACU-GUGGTT	959	CCACAGU-UAGCUGGA-GAUGTT	960	AD-15402				27		
2878-2896	GCUCCCUGA-UUAAUGGAG-GTT	961	CCUCCAU-UAAUCAG-GGAGCTT	962	AD-15293				27		
2881-2899	CCCUGAU-UAAUGGAG-GCUUTT	963	AAGCCUCCAU-UAAUCAG-GGTT	964	AD-15351				14		
2882-2900	CCUGAU-UAAUGGAG-GCUUATT	965	UAAGCCUCCA-UUAAUCAG-GTT	966	AD-15403				11		
2884-2902	UGAUUAAUG-GAGGCU-UAGCTT	967	GCUAAGCCUC-CAUUAAUCATT	968	AD-15404				38		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2885-2903	GAUUAAUG-GAGGCU-UAGCUTT	969	AGCUAAGCCU CCAUUAAUCTT	970	AD-15207				15		
2886- 2904	AUUAAUGGAG-GCUUAGCU-UTT	971	AAGCUAAGCC UCCAUUAAUTT	972	AD-15352				23		
2887-2905	UUAAUGGAG-GCUUAGCUU-UTT	973	AAAGCUAAGC CUCCAUUAATT	974	AD-15255				31		
2903-2921	UUUCUG-GAUG-GCAUCUAGCT sT	975	GCUA-GAUGCCAUC-CAGAAATsT	976	AD-9603		123				
2903-2921	uuucuGGAuG-GcAucuACcTsT	977	GCuA-GAUGCcAUC-cAGAAATsT	978	AD-9729		56				
2904-2922	UUCUGGAUG-GCAUCUAGCC TsT	979	GGCUA-GAUGCCAUC-CAGAATsT	980	AD-9599		139				
2904-2922	uucuGGAuG-GcAucuAGccTs T	981	GGCuA-GAUGCcAUC-cAGAATsT	982	AD-9725		38				
2905-2923	UCUGGAUG-GCAUCUAGCC ATsT	983	UGGCUA-GAUGCCAUC-CAGATsT	984	AD-9621		77				
2905-2923	ucuGGAuG-GcAucuAGccAT sT	985	UGGCuA-GAUGCcAUC-cAGATsT	986	AD-9747		63				

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2925-2943	AGGCUGGA-GACAG-GUGCGCTT	987	GCGCACCU-GUCUC-CAGCCUTT	988	AD-15405				32		
2926-2944	GGCUGGA-GACAG-GUGCGCCTT	989	GGCGCACCU-GUCUC-CAGCCTT	990	AD-15353				39		
2927-2945	GCUGGA-GACAG-GUGCGCCCTT	991	GGGCGCAC-CUGUCUC-CAGCTT	992	AD-15354				49		
2972-2990	UUCCU-GAGCCACCUU-UACUTT	993	AGUAAAGGUG-GCUCAG-GAATT	994	AD-15406				35		
2973-2991	UCCU-GAGCCUU-UACUCTT	995	GAGUAAAG-GUGGCUCAG-GATT	996	AD-15407				39		
2974-2992	CCUGAGCCAC-CUUUACU-CUTT	997	AGAGUAAAG-GUGGCUCAG-GTT	998	AD-15355				18		
2976-29[9]4	UGAGCCACCU-UUACU-CUGCTT	999	GCAGA-GUAAAGGUG-GCUCATT	100Q	AD-15356				50		
2978-2996	AGCCACCUU-UACU-CUGCUCTT	1001	GAGCAGA-GUAAAGGUG-GCUTT	1002	AD-15357				54		
2981-2999	CACCUUUACU-CUGCU-CUAUTT	1003	AUAGAGCAGA-GUAAAG-GUGTT	1004	AD-15269				23		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
2987-3005	UACUCUGCU-CUAUGCCAG-GTsT	1005	CCUGGCAUA-GAGCAGA-GUATsT	1006	AD-9565		74				
2987-3005	uAcucuGcu-cuAuGccAG-GTsT	1007	CCUGGcAuA-GAGcAGA-GuATsT	1008	AD-9691		49				
2998-3016	AUGCCAG-GCUUU-UCUAGCATT	1009	UGCUAG-CACAGCCUG-GCAUTT	1010	AD-15358				12		
3003-3021	AGGCU-GUGCUAG-CAAC ACCTT	1011	GGUGU-UGCUAG-CACAGCCUTT	1012	AD-15359				24		
3006-3024	CUGUGCUAG-CAACAC-CCAATT	1013	UUGGCUGU-UGCUAG-CACAGTT	1014	AD-15360				13		
3010-3028	GCUAG-CAACAC-CCAAAGGUTT	1015	ACCUUUG-GGUGU-UGCUAGCTT	1016	AD-15219				19		
3038-3056	GGAGCCAU-CACCUAG-GACUTT	1017	AGUCCUAG-GUGAUG-GCUCCTT	1018	AD-15361				24		
3046-3064	CACCUAG-GACU-GACUCGGCTT	1019	GCCGAGU-CAGUCCUAG-GUGTT	1020	AD-15273				36		
3051-3069	AGGACU-GACUCG-GCAGUGUTT	1021	ACACUGCCGA GUCAGUC-CUTT	1022	AD-15362				31		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3052-3070	GGACU-GACUCG-GCACUGUGTT	1023	CACACUGCCG AGUCAGUC-CTT	1024	AD-15192				20		
3074-3092	UGGUGCAUG-CACUGUCU-CATT	1025	UGA-GACAGUG-CAUGCAC-CATT	1026	AD-15256				19		
30A0-3098	AUGCACU-GUCU-CAGCCAACTT	1027	GUUGGCUGA-GACAGUG-CAUTT	1028	AD-15363				33		
3085-3103	CUGUCU-CAGCCAAC-CCGCUTT	1029	AGCGGGUUG-GCUGAGA-CATT	1030	AD-15364				24		
3089-3107	CUCAGCCAAC-CCGCUC-CACTsT	1031	GUGGAGCG-GGUUGGCU-GAGTsT	1032	AD-9604	35	49				
3089-3107	cucAGccAAc-ccGcuccAcTsT	1033	CUGCAC-CGCGUUC-CCUGAGTsT	1034	AD-9730		85				
3093-3111	CCCAAC-CCGCUCCAC-UACCTsT[T]	1035	GGUAGUG-GAGCGGGU-UGGCTsT	1036	AD-9527		45				
3093-3111	GccAAcccGcuc-cAcuAccTsT	1037	GGuAGUG-GAGCGGGU-UGGCTsT	1038	AD-9653		86				
3096-3114	AACCCCGUC-CACUACCCG-GTT	1039	CCG-GGUAGUC-GAGCGGGU-UTT	1040	AD-15365				62		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3099-3117	CCGCUCCAC-UACCCG-GCAGTT	1041	CUGCCG-GGUAGUG-GAGCGGTT	1042	AD-15294				30		
3107-3125	CUACCCG-GCAG-GGCUACA-CATT	1043	UGUGUAC-CCUGCCG-GGUACTT	1044	AD-15173				12		
3108-3126	UACCCG-GCAG-GGUACACAUT T	1045	AUGUGUAC-CCUGCCG-GUATT	1046	AD-15366				21		
3109-3127	ACCCGGCAG-GGUACACAU-UTT	1047	AAUGUGUAC-CCUGCCCG-GUTT	1048	AD-15367				11		
3110-3128	CCCGGCACG-GGUACACAU-UCTT	1049	GAAUGUGUAC-CCUGCCG-GGTT	1050	AD-15257				18		
3112-3130	CGGCAG-GGUACACAU-UCGCT	1051	GCGAAUGU-GUAC-CCUGCCGTT	1052	AD-15184				50		
3114-3132	GCAC-CCUACACAU-UCUCACTT	1053	GUGCGAAU-GUGUAC-CCUGCTT	1054	AD-15185				12		
3115-3133	CAG-GGUACACAU-UCGCACCTT	1055	GGUGCGAAU-GUGUAC-CCUGTT	1056	AD-15258				73		
3116-3134	AGGGUACACA-UUCGCAC-CCTT	1057	GGGUGCGAAU GUGUAC-CCUTT	1058	AD-15186				36		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3196-3214	GGAACU-GAGCCA-GAAACGCTT	1059	GCGUUUCUG-GCUCAGUUC-CTT	1060	AD-15274				19		
3197-3215	GAACU-GAGCCA-GAAACGCATT	1061	UGCGUU-UCUGGCU-CAGUUCTT	1062	AD-15368				7		
3198-3216	AACU-GAGCCACAAA CGCAGTT	1063	CUGCGUU-UCUGGCU-CAGUUTT	1064	AD-15369				17		
3201-3219	UGAGCCACAA ACGCACAU-UTT	1065	AAUCUGCUU-UCUGGCU-CATT	1066	AD-15370				19		
3207-3225	AGAAACGCA-GAUUG-GGCUGTT	1067	CAGCCCAAUC UGCGUU-UCUTT	1068	AD-15259				38		
3210-3228	AACGCAGAU-UGGGCUG-GCUTT	1069	AGCCAGCCCA AUCUGCGU-UTT	1070	AD-15408				52		
3233-3251	AGCCAAGCCU-CUUCU-UACUTsT	1071	AGUAAGAA-GAGGCUUG-GCUTsT	1072	AD-9597		23	21		0,04	
3233-3251	AGccAAGccucu-ucuuAcuTsT	1073	AGuAAGAA-GAGGCUUG-GCUTsT	1074	AD-9723		12	26			
3233-3251	AfgCfcAfaGfc-CfuCfuUfuAf-cUfTsT	1075	p-aGfuAfaGfaAf-gAfgGfcUfuGf-gCfuTsT	1076	AD-14680				15		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3233-3251	AGCfCTAAGCf CfCfUfCfU-fUfCACfUfTsT	1077	AGUfAAGAA-GAGGCfUfUfG-GCfUfTsT	1078	AD-14690				18		
3233-3251	AgCcAaGcCuC-uUcUuAcUTsT	1079	p-aGfuAfaGfaAf-gAfgGfcUfuGf-gCfuTsT	1080	AD-14700				15		
3233-3251	AgCcAaGcCuC-uUcUuAcUTsT	1081	AGUTAAGAA-GAGGCfUUfG-GCfUfTsT	1082	AD-14710				15		
3233-3251	AfgCfcAfaGfc-CfuCfuUfcU-fuAfcUTTsT	1083	AGUAAgaAGag-GCUUGgcuTsT	1084	AD-14720				18		
3233-3251	AGCfC-fAAGCfCfUfCfU-fUfCfUfUfAC-fUfTsT	1085	AGUAAgaAGag-GCUUGgcuTsT	1086	AD-14730				18		
3233-3251	AgCcAaGcCuC-uUcUuAcUTsT	1087	AGUAAgaAGag-GCUUGgcuTsT	1088	AD-14740				17		
3233-3251	UfgGfuUfcCfcUf-gAfgGfaCfcAf-gCfTsT	1089	p-gCfuCfgUfc-CfuCfaGfgG-faAfcCfaTsT	1090	AD-15086				85		
3233-3251	UfGGU-fUfCfCfCfUf-GAGGACfCf-AGCfTsT	1091	GCfUfGGUfCfC-fUfCfAg-ggaacFcTsT	1092	AD-15096				70		
3233-3251	UgCuUcCcUgA-gUaCcAgCTsT	1093	p-gCfuGfgUfc-CfuCfaGfaGfgG-faAfcCTaTsT	1094	AD-15106				71		

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position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3233-3251	UgGuUcCcU-gAgGaC-cAgCTsT	1095	GCfUfGGUfCfC-fUfCfAG-GGAACfCfATsT	1096	AD-15116				73		
3233-3251	UfgGfuUfcCT-cUfgAfgGfaCf-cAfgCfTsT	1097	GCUGGucCU-caGGGAAc-caTsT	1098	AD-15126				71		
3233-3251	UfGGU-fUfCfCfCfUf-GAGGACfCf-AGCfTsT	1099	GCUGGncCU-caGGGAAc-caTsT	1100	AD-15136				56		
3233-3251	UgGuUcCcU-gAgGaC-cAgCTsT	1101	GCUGGucCU-caGGGAAc-caTsT	1102	AD-151146				72		
3242-3260	UCUUCUUACU-UCACCCG-GCTT	1103	GCCGGGU-GAAGUAAGAA-GATT	1104	AD-15260				79		
3243-3261	CUUCUUACUU-CACCCG-GCUTT	1105	AGCCGUGU-GAAGUAA-GAAGTT	1106	AD-15371				24		
3244-3262	UUCUUACUUC-CACCCG-GCUGTT	1107	CAGCCGGGU-GAAGUAA-GAATT	1108	AD-15372				52		
3262-3280	GGGCUCCU-CAUUUUUACG-GTT	1109	CCGUAAAAAU-GAG-GAGCCCTT	1110	AD-15172				27		
3263-3281	GGCUCCUCA-UUUUUACG-GTT	1111	CCCGUAAAAA UGAG-GAGCCTT	1112	AD-15295				22		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3264-3282	GCUCCUCAUU-UUUACG-GGUTT	1113	AC-CCGUAAAAAU-GAGGAGCTT	1114	AD-15373				11		
3265-3283	CUCCUCAUUU-UUACG-GGUATT	1115	UAC-CCGUAAAAAU-GAGGAGTT	1116	AD-15163				18		
3266-3284	UCCUCAUUUU-UACCG-GUAATT	1117	UUAC-CCGUAAAAAU-GAGGATT	1118	AD-15165				13		
3267-3285	CCUCAUUUU-UACG-GGUAACTT	1119	GUUAC-CCGUAAAAAU-GAGGTT	1120	AD-15374				23		
3268-3286	CUCAUUUU-UACGGGUAA-CATT	1121	UGUUAC-CCGUAAAAAU GTT	1122	AD-15296				13		
3270-3288	CAUUUUUACG-GGUAACAGUT T	1123	ACUGUUAC-CCGUAAAAAU GTT	1124	AD-15261				20		
3271-3289	AUUUUUACG-GGUAACAGUG TT	1125	CACUGUUAC-CCGUAAAAAU TT	1126	AD-15375				90		
3774-3292	UUUACG-GGUAACAGU-GAGGTT	1127	CCUCACUGU-UAC-CCGUAAATT	1128	AD-15262				72		
3308-3326	CAGACCAG-GAAGCUCG-GUGTT	1129	CACCGAGCU-UCCUG-GUCUGTT	1130	AD-15376				14		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3310-3328	GACCAG-GAAGCUCG-GUGAGTT	1131	CUCAC-CGAGCUUC-CUGGUCTT	1132	AD-15377				19		
3312-3330	CCAG-GAAGCUCGCU GAGUCTT	1133	CACUCAC-CGAGCUUC-CUGGTT	1134	AD-15409				17		
3315-3333	GGAAGCUCG-GUGAGU-GAUGTT	1135	CAUCACUCAC-CGAGCUUC-CTT	1136	AD-15378				18		
3324-3342	GUGAGU-GAUGGCA-GAACGATT	1137	UCGU-UCUGCCAU-CACUCACTT	1138	AD-15410				8		
3326-3344	GAGUGAUG-GCGAAC-GAUGTT	1139	CAUCUUUCUC-CCAU-CACUCTT	1140	AD-15379				11		
3330-3348	GAUGGCA-GAAC-GAUGCCUGTT	1141	CACCCAUCCU-UCUC-CCAUCTT	1142	AD-15187				36		
3336-3354	ACAAC-CAUGCCUC-CAGCCATT	1143	UGCCUGCAG-GCAUCGU-UCUTT	1144	AD-15263				18		
3339-3357	AC-GAUGCCUG-CAGGCAUG-GTT	1145	CCAUGCCUG-CAG-GCAUCGUTT	1146	AD-15264				75		
3348-3366	GCAGGCAUG-GAACUUUU-UCTT	1147	GAAAAAGUUC-CAUGCCUGCT T	1148	AD-15297				21		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3356-3374	GGAACUUUU-UCCGUUAU-CATT	1149	UGAUAACG-GAAAAAGUUC-CTT	1150	AD-15208				6		
3357-3375	GAACUUUUUC-CGUUAU-CACTT	1151	GUGAUAACG-GAAAAAGU-UCTT	1152	AD-15209				28		
3358-3376	AACUUUUUC-CUUUAUCAC-CTT	1153	GGU-GAUAACG-GAAAAAGUUTT	1154	AD-15193				131		
8370-3388	UAUCACCCAG-GCCUUAU-UCTT	1155	GAAUCAG-GCCUGGGU-GAUATT	1156	AD-15380				88		
3378-3396	AGGCCUGAU-UCACUG-GCCUTT	1157	AGGCCAGU-GAAUCAG-GCCUTT	1158	AD-15298				43		
3383-3401	UGAUUCACUG-GCCUGGCG-GTT	1159	CCGCCAG-GCCAGUGAAU-CATT	1160	AD-15299				99		
3385-3403	AUUCACUG-GCCUGGCG-GAGTT	1161	CUCCGCCAG-GCCAGU-GAAUTT	1162	AD-15265				95		
3406-3424	GCUUCUAAG-GCAUGGUCG-GTT	1163	CCGAC-CAUGCCUUA-GAAGCTT	1164	AD-15381				18		
3407-3425	CUUCUAAG-GCAUGGUCG-GGTT	1165	CCCGAC-CAUGCCUUA-GAAGTT	1166	AD-15210				40		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3429-3447	GAG-GGCCAACAAC UGUCCCTT	1167	GGGACAGUU-GUUG-GCCCUCTT	1168	AD-15270				83		
3440-3458	ACUGUCCCUC-CUUGAG-CACTsT	1169	GUGCUCAAG-GAG-GGACAGUTsT	1170	AD-9591	75	95				
3440-3458	AcuGucccuccuu-GAGcAcTsT	1171	GUUCUcAAG-CACCGAcAU-UTsT	1172	AD-9717		105				
3441-3459	CUUUCCCUC-CUDCAGCAC-CTsT	1173	GGUGCU-CAAGGAG-GGACAGTsT	1174	AD-9622		94				
3441-3459	cuGucccuccuu-GAGcAccTsT	1175	GGUGCUcAAG-GAG-GGAcAGTsT	1176	AD-9748		103				
3480-3498	ACAUUAUCUU-UUG-GGUCUTsT	1177	AGACCCAAAA-GAUAAAU-GUTsT	1178	AD-9587		63	49			
3480-3498	AcAuuuAucuuu-uGGGucuTsT	1179	AGACCcAAAA-GAuAAAU-GUTsT	1180	AD-9713		22	25			
3480-3498	AfcAfuUfuAfuCf-uUfuUfgGfgUf-cUfTsT	1181	p-aGfaCfcCfaA-faAfgAfuA-faAfuGfuTsT	1182	AD-14679				19		
3480-3498	ACfAUfUfU-fAUfCfUfUfUfU-fUfGGGUfCfC-fUfTs T	1183	AGACfCfC-fAAAAGAU-fAAAUfGUfTsT	1184	AD-14689				24		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3480-3498	AcAuUuAuCuU-uUgGgUcUTsT	1185	p-aGfaCfcCfaA-faAfgAfuA-faAfuCfuTsT	1186	AD-14699				19		
3480-3498	AcAuUuAuCuU-uUgGgUcUTsT	1187	AGACfCfC-fAAAAGAU-fAAAUfGUfTsT	1188	AD-14709				21		
3480-3498	AfcAfuUfuAfuCf-uUfuUfgGfgUf-cUfcUfTsT	1189	AGACCcaAAa-gAUAAAuguTsT	1190	AD-14719				24		
3480-3498	ACfAUfUfU-fAUfCfUfUfUfUf-GGGUfTUTs T	1191	AGACCcaAAa-gAUAAAuguTsT	1192	AD-14729				23		
3480-3498	AcAuUuAuCuU-uUgGgUcUTsT	1193	AGACCcAAa-gAUAAAuguTsT	1194	AD-14739				24		
3480-3498	GfcCfaUfcUf-gCfuGfcCfgGf-aGfcCfTsT	1195	p-gCfcUfcCfgGf-cAfgGfaUfgUfg-GfcTsT	1196	AD-15085				74		
3480-3498	GCfCfAUfCfU-fUfGCfGCfCfG-CAGCTCfTsT	1197	GGCfUfCfCfG-GCfAGCf-AGAUfGGCfTsT	1198	AD-15095				60		
3480-3498	GcCaUcUgCuG cCgGaGcCTsT	1199	p-gGfcUfcCfcCf-gGfcAfgCfaG-faUfgGfcTsT	1200	AD-15105				33		
3480-3498	GcCaUcUgCuG cCgGaGcCTsT	1201	CGCfUfCfGGCf-AGCfAGUf-GCfTsT	1202	AD-*15115*				30		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3480-3498	GfcCfaUfcUf-gCfuGfcCfgGf-aGfcCfTsT	1203	GGCUCauG-CagCAGAUg-gcTsT	1204	AD-15125				54		
3480-3498	GCfCfAUfCfUf-GCfGCfCfCfG-CAGCTCTTsT	1205	GGCUCauG-CagCAGUg-gcTsT	1206	AD-15135				51		
3480-3498	GcCaUcUgCu-UcCgUaUcCTsT	1207	GGUCUCauC-CagCAGAUg-gcTsT	1208	AD-15145				49		
3481-3499	CAUUUAUCUU-UUG-GGUCUGTsT	1209	CAGAC-CCAAAACAUAA AUCTsT	1210	AD-9578	49	61				
3481-3499	cAuuuAucuuu-uCGUucuGTsT	1211	cAGAC-CcAAAAUAuAA AUGTsT	1212	AD-9704		111				
3485-3503	UAUCUUUUG-GGUGUGUC-CTsT	1213	AGGACAAAC-CCAAAA-GAUATsT	1214	AD-9558		66				
3485-3503	uAucuuuuG-GGucuGuc-cuTsT	1215	AGGAcAGAC-CcAAAA-GAuATsT	1216	AD-9684		63				
3504-3522	CUCUGU-UGCUUU-UACAGTsT	1217	CUGUAAAAAG-GCAACAGATsT	1218	AD-9634		29	30			
3504-3522	cucuGuuGccuu-uuuAcAGTsT	1219	CUGuAAAAAG-GcAAcA-GAGTsT	1220	AD-9760		14	27			

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3512-3530	CCUUUU-UACAGCCAAC-UUUTT	1221	AAAGUUGGCU-GUAAAAAG-GTT	1222	AD-15411				5		
3521-3539	AGCCAACUUU-UCUAGAC-CUTT	1223	AGGUCUA-GAAAAGUUG-GCUTT	1224	AD-15266				23		
3526-3544	ACUUUUCUA-GACCUGUUU-UTT	1225	AAAACAG-GUCUA-GAAAAGUTT	1226	AD-15382				12		
3530-3548	UUCUAGACCU-GUUUUGCU-UTsT	1227	AAG-CAAAACAG-GUCUAGAATsT	1228	AD-9554		23	24			
3530-3548	uucuAGAccuGu-uuuGcuuTsT	1229	AAGcAAAAcAG-GUCuAGAATsT	1230	AD-9680		12	22		0,10	0,10
3530-3548	UfuCfuAfgAfc-CfuGfuUfuUf-gCfuUfTsT	1231	p-aAfgCfaAfaAf-cAfgGfuCfuAf-gAfaTsT	1232	AD-14676				12		
3530-3548	UfUfCfUf-AGACfCfUfGU-fUfUfUfGCfU-fUfTs T	1233	AAGCfAAAACf-AGGUfCfUf-AGAATsT	1234	AD-14686				13		
3530-3548	UuCuAgAcCuG-uUuUgCuUTsT	1235	p-aAfgCfaAfaAf-cAfgGfuCfuAf-gAfaTsT	1236	AD-14696				12		
3530-3548	UuCuAgAcCuG-uUuUgCuUTsT	1237	AGCfAAAACf-AGGUfCfUf-AGAATsT	1238	AD-14706				18		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3530-3548	UfuCfuAfgAfc-CfuGfuUffCfu-UfTsT	1239	AAGcAaaACag-GUCUAgaaTsT	1240	AD-14716				17		
3530-3548	UfUfCfUf-AGACfCfUfGU-fUfUfUfGCfU-fUfTs T	1241	AAGcAaaACag-GUCUAgaaTsT	1242	AD-14726				16		
3530-3548	UuCuAgAcCuG-uUuUgCuUTsT	1243	AAGcAaaACag-GUCUAgaaTsT	1244	AD-14736				9		
3530-3548	CfaUfaGfgCfcUf-gGfaGfuUfuU-fuAfuUfTsT	1245	p-aAfuAfaAf-cUfcCfaGfgCfc-UfaUfgTsT	1246	AD-15082				27		
3530-3548	CfAUfAGGCfC-fUfGGAGUfUfU-FAUfUfTsT	1247	AAUfAAAC-fUfCfCfAG-GCfCfAGGCfCf-UfAUfGTsT	1248	AD-15092				28		
3530-3548	CaUaGgCcUg-GaGuUuAu-UTsT	1249	p-aAfuAfaAf-cUfcCfaGfgCfc-UfaUfgTsT	1250	AD-15102				19		
3530-3548	CaUaGgCcUg-GaGuUuAu-UTsT	1251	AAUfAAACfU-fUfCfCfAGGCfC-fUfAUfGTs	1252	AD-15112				17		
3530-3548	CfaUfaGtgCfcUf-gGfaGfuUfuAfu-UfTsT	1253	AAUAAacUC-caG-GCCUaugTsT	1254	AD-15122				56		
3530-3548	CfAUfAGGCfC-fUfGGAGUfUfU-fAUfUfTsT	1255	AAUAAacUC-caG-GCCUaugTsT	1256	AD-15132				39		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3530-3548	CaUaGgCcUg-GaGuUuAu-UTsT	1257	AAUAAacUC-caG-GCCUaugTsT	1258	AD-15142				46		
3531-3549	UCUAGACCUG-UUUUGCUU-UTsT	1259	AAAG-CAAAACAG-GUCUAGATst	1260	AD-9553		27	22		0,02	
3531-3549	ucuACAccuGuu-uuGcuuuTsT	1261	AAAGcAAAcAG-GUCuAGATsT	1262	AD-9679		17	21			
3531-3549	UfcUfaGfaCfcUf-gUfuUfuGfcUfu-UfTsT	1263	p-aAfaGfcAfaA-faCfaGfgUfcUf-aGfaTsT	1264	AD-14675				11		
3531-3549	UfCfUfAGACfC-fUfGUfUfUfUf-GCfUfUfUfUfTs T	1265	AAAGCfAAAACf AGGUfCfUf-AGATst	1266	AD-14685				19		
3531-3549	UcUaGaCcUgU-uUuGcUuUTsT	1267	p-aAfaGfcAfaA-faCfaGfgUfcUf-aGfaTst	1268	AD-14695				12		
3531-3549	UcUaGaCcUgU-uUuGcUuUTsT	1269	AAAGCfAAAACf GGUfCfUf-AGATsT	1270	AD-14705				16		
3531-3549	UfcUfaGfaCfcUf-gUfuUfuGfcUfu-UfTsT	1271	AAAG-CaaAAcaG-GUCUagaTsT	1272	AD-14715				19		
3531-3549	UfCfUfAGACfC-fUfGUfUfUfUf-GCfUfUfUfUfTs T	1273	AAAG-CaaAACaG-GUCUagaTsT	1274	AD-14725				19		

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3531-3549	UcUaGaCcUgU-uUuGcUuUTsT	1275	AAAG-CaaAAcaG-GUCUagaTsT	1276	AD-14735				19		
3531-3549	UfcAfuAfgGfc-CfuGfgAfgUfuU-faUfTsT	1277	p-aUfaAfaCfuCf-cAfgGfc-CfuAfuGfaTsT	1278	AD-15081				30		
3531-3549	UfCfAUfGGCfC-fUfGGAGUfUfU-fAUfTsT	1279	AUfAAAC-fUfCfCfAGGCfC-fUfAUfGATsT	1280	AD-15091				16		
3531-3549	UcAuAgGcCuG-gAgUuUaUTsT	1281	p-aUfaAfaCfuCf-cAfgGfc-CfuAfuGfaTsT	1282	AD-15101				16		
3531-3549	UcAuAgGcCuG-gAgUuUaUTsT	1283	AUfAAAC-fUfCfCfAGGCfC-fUfAUfGATsT	1284	AD-15111				11		
3531-3549	UfcAfuAfgGfc-CfuGfgAfgUfuU-faUTsT	1285	AUAAAcuCCag-GCCUAugaTsT	1286	AD-15121				19		
3531-35549	UfCfAUfAG-GCfCfUfG-GAGUfUfU-fAUfTsT	1287	AUAAAcuCCag-GCCUAugaTsT	1288	AD-15131				17		
3531-3549	UcAuAgGcCuG-gAgUuUaUTsT	1289	AUAAAcuCCag-GCCUAugaTsT	1290	AD-15141				18		
3557-3575	UGAAGAUAUU-UAUUCUG-GGTsT	1291	CCCA-GAAUAAAUAU CUUCATsT	1292	AD-9626		97	68			

(continued)

position in human access. # NM_174936	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Duplex name	Mean percent remaining mRNA transcript at siRNA concentration/ in cell type				IC50 in HepG 2 [nM]	IC50 in Cynomol-gous monkey Hepatocyte [nM]s
						100 nM/ HepG 2	30 nM/ HepG 2	3nM/ HepG 2	30 nM/ Hel a		
3557-3575	uGAAGAuAuu-uAuucuGGGTsT	1293	CCcA-GAAuAAAuAUCUUcATsT	1294	AD-9752		28	33			
3570-3588	UCUGGGUUU-UGUAGCAUU-UTsT	1295	AAAUGCUACAAAACCCA-GATsT	1296	AD-9629		23	24			
3570-3588	ucuGGGuuuu-GuAGcAuuuTsT	1297	AAAUGCuAcAAAACCcAGATsT	1298	AD-9755		28	29			
3613-3631	AUAAAAACAAACAAACGUUTT	1299	AACGUUUGUU-UGUUUU-UAUTT	1300	AD-15412				21		
3617-3635	AAACAAACAAACGUUGUCCTT	1301	GGACAACGUU-UGUUUGUUU-UTT	1302	AD-15211				73		
3618-3636	AACAAACAAACGUUGUCCUTT	1303	AGGACAACGU-UUGUUUGU-UTT	1304	AD-15300				41		

[1] U, C, A, G: corresponding ribonucleotide; T: deoxythymidine; u, c, a, g: corresponding 2'-*O*-methyl ribonucleotide; Uf, Cf, Af, Gf: corresponding 2'-deoxy-2'-fluoro ribonucleotide; where nucleotides are written in sequence, they are connected by 3'-5' phosphodiester groups; nucleotides with interjected "s" are connected by 3'-*O*-5'-*O* phosphorothiodiester groups; unless denoted by prefix "p-", oligonucleotides are devoid of a 5'-phosphate group on the 5'-most nucleotide; all oligonucleotides bear 3'-OH on the 3'-most nucleotide

Table 2.

Duplex number	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Remaining mRNA in % of controls at siRNA conc. of 30 nM
AD-10792	GccuGGAGuuuAuucGGAATsT	1305	UUCCGAAuAAACUCcAGGCTsT	1306	15
AD-10793	GccuGGAGuuuAuucGGAATsT	1307	uUcCGAAuAAACUccAGCCTsT	1308	32
AD-10796	GccuGGACuuuAuucGGAATsT	1309	UUCCGAAUAAACUCCAGGCTsT	1310	13
AD-12038	GccuGGAGuuuAuucGGAATsT	1311	uUCCGAAUAAACUCCAGGCTsT	1312	13
AD-12039	GccuGGAGuuuAuucGGAATsT	1313	UuCCGAAUAAACUCCAGGCTsT	1314	29
AD-12040	GccuGCAGuuuAuucGGAATsT	1315	UUcCGAAUAAACUCCAGGCTsT	1316	10
AD-12041	GccuGGAGuuuAuucGGAATsT	1317	UUCcGAAUAAACUCCAGGCTsT	1318	11
AD-12042	GCCUGGAGUUUAUUCGGAATsT	1319	uUCCGAAUAAACUCCAGGCTsT	1320	12
AD-12043	GCCUGGAGUUUAUUCGGAATsT	1321	UuCCGAAUAAACUCCAGGCTsT	1322	13
AD-12044	GCCUGGAGUUUAUUGGGAATsT	1323	UUcCGAAUAAACUCCACGGCTsT	1324	7
AD-12045	GCCUGGAGUUUAUUCGGAATsT	1325	UUCcGAAUAAACUCCCAGGCTsT	1326	8
AD-12046	GccuGGAGuuuAuucGGAA	1327	UUCCGAAUAAACUCCAGGCscsu	1328	13
AD-12047	GccuGGAGuuuAuucGGAAA	1329	UUUCCGAAUAAACUCCAGGCscsu	1330	17
AD-12048	GccuGGAGuuuAuucGGAAAA	1331	UUUUCCGAAUAAACUCCAGGCscsu	1332	43
AD-12049	GccuGGAGuuuAuucGGAAAAG	1333	CUUUUCCGAAUAAACUCCAGGCscsu	1334	34
AD-12050	GccuGGAGuuuAuucGGAATTab	1335	UUCCGAAUAAACUCCAGGCTTab	1336	16
AD-12051	GccuGGAGuuuAuucGGAAATTab	1337	UUUCCGAAuAAACUCCAGGCTTab	1338	31
AD-12052	GccuGGAGuuuAuucGGAAAATTab	1339	UUUUCCGAAUAAACUCCAGGCTTab	1340	81
AD-12053	GccuGGAGuuuAuucGGAAAAGTTab	1341	CUUUUCCGAAUAAACUCCAGGCTTab	1342	46
AD-12054	GCCUGGAGUUUAUUCGGAATsT	1343	UUCCGAAUAAACUCCAGGCscsu	1344	8
AD-12055	GccuGGAGuuuAuucGGAATsT	1345	UUCCGAAUAAACUCCAGGCscsu	1346	13
AD-12056	GcCuGgAgUuUaUuCgGaA	1347	UUCCGAAUAAACUCCAGGCTTab	1348	11
AD-12057	GcCuGgAgUuUaUuCgGaA	1349	UUCCGAAUAAACUCCAGGCTsT	1350	8
AD-12058	GcCuGgAgUuUaUuCgGaA	1351	UUCCGAAuAAACUCcAGGCTsT	1352	9
AD-12059	GcCuGgAgUuUaUuCgGaA	1353	uUcCGAAuAAACUccAGGCTsT	1354	23

(continued)

Duplex number	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Remaining mRNA in % of controls at siRNA conc. of 30 nM
AD-12060	CcCuGgAgUuUaUuCgGaA	1355	UUCCGaaUAaaCUCCAggc	1356	10
AD-12061	GcCuGgnAgUuUaUuCgGaATsT	1357	UUCCGaaUAaaCUCCAggcTsT	1358	7
AD-12062	GcCuGgAgUuUaUuCgGaATTab	1359	UUCCGaaUAaaCUCCAggcTTab	1360	10
AD-12063	GcCuGgAgUuUaUuCgGaA	1361	UUCCGaaaUAaaCUCCAggcscsu	1362	19
AD-12064	GcCuGgnAgUuUaUuCgGaATsT	1363	UUCCGAAuAAACUCcAGGCTsT	1364	15
AD-12065	GcCuGgAgUuUaUuCgGaATTab	1365	UUCCGAAuAAACUCcAGGCTTab	1366	16
AD-12066	GcCuGgAgUuUaUuCgGaA	1367	UUCCGAAuAAACUCcAGGCscsu	1368	20
AD-12067	GcCuGgnAgUuUaUuCgGaATsT	1369	UUCCGAAUAAACUCCAGGCTsT	1370	17
AD-12068	GcCuGgAgUuUaUuCgGaATTab	1371	UUCCGAAUAAACUCCAGGCTTab	1372	18
AD-12069	GcCuGgAgUuUaUuCgCGA	1373	UUCCGAAUAAACUCCAGGCscsu	1374	13
AD-12338	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1375	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfc	1376	15
AD-12339	GcCuGgAgUuUaUuCgGaA	1377	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfc	1378	14
AD-12340	GccuGGAGuuuAuucGGAA	1379	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfc	1380	19
AD-12341	GfcCfuGfgAfgUfuUfaUfuCfGfaAfTsT	1381	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTsT	1382	12
AD-12342	GfcCfuGfgAfgAfgUfuUfaUfuCfgGfaAfTsT	1383	UUCCGAAuAAACUCcAGGCTsT	1384	13
AD-12343	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTsT	1385	uUcCGAAuAAACUccAGGCTsT	1386	24
AD-12344	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTsT	1387	UUCCGAAUAAACUCCAGGCTsT	1388	9
AD-12345	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTsT	1389	UUCCGAAUAAACUCCAGGCscsu	1390	12
AD-12346	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTsT	1391	UUCCGaaUAaaCUCCAggcscsu	1392	13
AD-12347	GCCUGGAGUUUAUUCGGAATsT	1393	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTsT	1394	11
AD-12348	GccuGGAGuuuAuucGGAATsT	1395	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTsT	1396	8
AD-12349	GcCuGgnAgUuUaUuCgGaATsT	1397	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTsT	1398	11
AD-12350	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTTab	1399	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTTab	1400	17
AD-12351	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1401	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcsCfsu	1402	11

(continued)

Duplex number	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Remaining mRNA in % of controls at siRNA conc. of 30 nM
AD-12352	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1403	UUCCGaaUAaaCUCCAggcscsu	1404	11
AD-12354	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1405	UUCCGAAUAAACUCCAGGCscsu	1406	11
AD-12355	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1407	UUCCGAAuAAACUCcAGGCTsT	1408	9
AD-12356	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1409	uUcCGAAuAAACUccAGGCTsT	1410	25
AD-12357	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1411	UUCCGaaUAaaCUCCAggc	1412	56
AD-12358	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1413	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfc	1414	29
AD-12359	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1415	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcsCfsu	1416	30
AD-12360	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1417	UUCCGAAUAAACUCCAGGCscsu	1418	15
AD-12361	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1419	UUCCGAAuAAACUCcAGGCTsT	1420	20
AD-12362	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1421	uUcCGAAuAAACUccAGGCTsT	1422	51
AD-12363	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1423	UUCCGaaUAaaCUCCAggcscsu	1424	11
AD-12364	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaAsT	1425	UCCGaaUAaaCUCCAggcTsT	1426	25
AD-12365	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaAsT	1427	UUCCGAAuAAACUCcAGGCTsT	1428	18
AD-12366	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaAsT	1429	UUCCGAAUAAACUCCAGGCTsT	1430	23
AD-12367	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1431	UUCCGaaUAaaCUCCAggcTsT	1432	42
AD-12368	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1433	UUCCGAAuAAACUCcAGGCTsT	1434	40

(continued)

Duplex number	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Remaining mRNA in % of controls at siRNA conc. of 30 nM
AD-12369	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1435	UUCCGAAUAAACUCCAGGCTsT	1436	26
AD-12370	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1437	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1438	68
AD-12371	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1439	P-UfUfCfCfGAAUfAAACfCfUfCfCfAGGCfsCfsUf	1440	60
AD-12372	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1441	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcsCfsu	1442	60
AD-12373	GmocmocmouGGAGmoumoumouAmoumoumocGGAATsT	1443	UUCCGAAUAAACUCCAGGCTsT	1444	55
AD-12374	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1445	UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1446	9
AD-12375	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1447	UUCCGAAUAAACUCCAGGCTsT	1448	16
AD-12377	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1449	uUcCGAAuAAACUccAGGCTsT	1450	88
AD-12378	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1451	UUCCGaaUAaaCUCCAggcscsu	1452	6
AD-12379	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1453	UUCCGAAUAAACUCCAGGCscsu	1454	6
AD-12380	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1455	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcsCfsu	1456	8
AD-12381	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1457	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcTsT	1458	10
AD-12382	GCfCfUfGGAGUfUfUfAUfUfCfGGAATsT	1459	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1460	7
AD-12383	GCCUGGAGUUUAUUCGGAATsT	1461	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1462	7
AD-12384	GccuGGAGuuuAuucGGAATsT	1463	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1464	8
AD-12385	GcCuGgnAgUuUaUuCgGaATsT	1465	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1466	8
AD-12386	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1467	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfTsT	1468	11
AD-12387	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1469	UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1470	13
AD-12388	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1471	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfc	1472	19
AD-12389	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1473	P-uUfcCfgAfaUfaAfaCfuCfcAfgGfcsCfsu	1474	16
AD-12390	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1475	UUCCGAAUAAACUCCAGGCscsu	1476	17

(continued)

Duplex number	Sense strand sequence (5'-3')[1]	SEQ ID NO:	Antisense-strand sequence (5'-3')[1]	SEQ ID NO:	Remaining mRNA in % of controls at siRNA conc. of 30 nM
AD-12391	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1477	UUCCGaaUAaaCUCCAggc	1478	21
AD-12392	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1479	UUCCGAAUAAACUCCAGGCTsT	1480	28
AD-12393	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1481	UUCCGAAuAAACUCcAGGCTsT	1482	17
AD-12394	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1483	uUcCGAAuAAACUccAGGCTsT	1484	75
AD-12395	GmocCmouGmogAmogUmouUmoaUmouCmogGmoaATsT	1485	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1486	55
AD-12396	GmocCmouGmogAm02gUmouUmoaUmouCmogGmoaA	1487	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1488	59
AD-12397	GfcCfuGfgAfgUfuUfaUfuCfgGfaAf	1489	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1490	20
AD-12398	GfcCfuGfgAfgUfuUfaUfuCfgGfaAfTsT	1491	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1492	11
AD-12399	GcCuGgnAgUuUaUuCgGaATsT	1493	P-UfUfCfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1494	13
AD-12400	GCCUGGAGUUUAUUCGGAATsT	1495	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1496	12
AD-12401	GccuGGAGuuuAuucGGAATsT	1497	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1498	13
AD-12402	GccuGGAGuuuAuucGGAA	1499	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1500	14
AD-12403	GCfCfUfGGAGGUfUfUfAUfUfCfGGAA	1501	P-UfUfCfCfGAAUfAAACfUfCfCfAGGCfsCfsUf	1502	4
AD-9314	GCCUGGAGUUUAUUCGGAATsT	1503	UUCCGAAUAAACUCCAGGCTsT	1504	9

[1] U, C, A, G: corresponding ribonueleotide; T: deoxythymidine; u, c, a, g: corresponding 2'-*O*-methyl ribonucleotide; Uf, Cf, Af, Gf: corresponding 2'-deoxy-2'-fluoro ribonucleotide; moc, mou, mog, moa: corresponding 2'-MOE nucleotide; where nucleotides are written in sequence, they are connected by 3'-5' phosphodiester groups; ab: 3'-terminal abasic nucleotide; nucleotides with interjected "s" are connected by 3'-*O*-5'-*O* phosphorothiodiester groups; unless denoted by prefix "p-", oligonucleotides are devoid of a 5'-phosphate group on the 5'-most nucleotide; all oligonucleotides bear 3'-OH on the 3'-most nucleotide

[0151] The invention furthermore comprises the following items:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human PCSK9 gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding PCSK9, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene.

2. The dsRNA of item 1, wherein said first sequence is selected from the group consisting of Tables 1 and 2 and said second sequence is selected from the group consisting of Tables 1 and 2.

3. The dsRNA of item 1, wherein said dsRNA comprises at least one modified nucleotide.

4. The dsRNA. of item 2, wherein said dsRNA comprises at least one modified nucleotide.

5. The dsRNA of item 3, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

6. The dsRNA of item 3, wherein said modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

7. The dsRNA of item 3, wherein said first sequence is selected from the group consisting of Tables 1 and 2 and said second sequence is selected from the group consisting of Tables 1 and 2

8. The dsRNA of item 6, wherein said first sequence is selected from the group consisting of Tables 1 and 2, and said second sequence is selected from the group consisting of Tables 1 and 2.

9. A cell comprising the dsRNA of item 1.

10. A pharmaceutical composition for inhibiting the expression of the PCSK9 gene in an organism, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding PCSK9, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene.

11. The pharmaceutical composition of item 10, wherein said first sequence of said dsRNA is selected from the group consisting of Tables 1 and 2, and said second sequence of said dsRNA is selected from the group consisting of Tables 1 and 2.

12. The pharmaceutical composition of item 10, wherein said first sequence of said dsRNA is selected from the group consisting of Tables 1 and 2 and said second sequence of said dsRNA is selected from the group consisting of Tables 1 and 2.

13. A method for inhibiting the expression of the PCSK9 gene in a cell, the method comprising:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNAs), wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding PCSK9, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript

of the PCSK9 gene, thereby inhibiting expression of the PCSK9 gene in the cell.

14. A method of treating, preventing or managing pathological processes which can be mediated by down regulating PCSK9 gene expression comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a dsRNA, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding PCSK9, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene.

15. A vector for inhibiting the expression of the PCSK9 gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is substantially complementary to at least a part of a mRNA encoding PCSK9 and wherein said dsRNA is less than 30 base pairs in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits the expression of said PCSK9 gene.

16. A cell comprising the vector of item 15.

17. A double-stranded ribonucleic acid (dsRNA) for reducing the expression level of a human PCSK9 gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding PCSK9, and wherein said dsRNA, upon contact with a cell expressing said PCSK9, reduces the expression level of said PCSK9 gene.

18. The dsRNA of item 17, wherein said contact reduces the expression level of said PCSK9 gene.

19. The dsRNA of item 17, wherein said contact is performed *in vitro* at 30 nM or less.

20. A pharmaceutical composition for reducing the expression level of the PCSK9 gene in an organism, comprising the dsRNA of item 17 and a pharmaceutically acceptable carrier.

21. A method of treating a PCSK9 associated disorder comprising administering to a patient in need of such treatment, a therapeutically effective amount of a dsRNAs of item 17.

22. A method of treating a PCSK9-associated disorder comprising administering to a patient in need of such treatment, a therapeutically effective amount of a dsRNA of item 17.

Claims

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human PCSK9 gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is fully complementary to at least a part of an mRNA encoding PCSK9, wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said PCSK9, inhibits expression of said PCSK9 gene by at least 40%, and wherein said part of an mRNA encoding PCSK9 consists of the sequence UCAUAGGCCUGGAGUUUAU.

2. The dsRNA of claim 1, wherein said dsRNA comprises at least one modified nucleotide.

3. The dsRNA of claim 1 or 2, wherein said modified nucleotide is chosen from the group of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

4. The dsRNA of claim 2 or 3, wherein said first sequence is the sequence of SEQ ID NO: 453 and said second

sequence is the sequence of SEQ ID NO: 454.

5. A pharmaceutical composition for inhibiting the expression of the PCSK9 gene in an organism, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA is as defined in any one of claims 1 to 4.

6. An in vitro method for inhibiting the expression of the PCSK9 gene in a cell, the method comprising:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA is as defined in any one of claims 1 to 4; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the PCSK9 gene, thereby inhibiting expression of the PCSK9 gene in the cell.

7. A dsRNA for treating, preventing or managing pathological processes which can be mediated by down regulating PCSK9 gene expression, wherein the dsRNA is as defined in any one of claims 1 to 4.

8. A vector for inhibiting the expression of the PCSK9 gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein said dsRNA is as defined in any one of claims 1 to 4.

9. An isolated cell comprising the dsRNA of any one of claims 1 to 4 or the vector of claim 8.

10. A double-stranded ribonucleic acid (dsRNA) for reducing the expression level of a human PCSK9 gene in a cell, wherein said dsRNA is as defined in any one of claims 1 to 4.

11. The dsRNA of claim 10, wherein said contact reduces the expression level of said PCSK9 gene.

12. The dsRNA of claim 10, wherein said contact is performed in vitro at 30nM or less.

13. A pharmaceutical composition for reducing the expression level of the PCSK9 gene in an organism, comprising the dsRNA of claim 10 and a pharmaceutically acceptable carrier.

14. The dsRNA of claim 10 for treating a PCSK9 associated disorder.

15. The dsRNA of claim 14, wherein said PCSK9 associated disorder is hyperlipidemia.

Patentansprüche

1. Doppelsträngige Ribonukleinsäure (dsRNA) zum Inhibieren der Expression eines menschlichen PCSK9-Gens in einer Zelle, wobei die dsRNA mindestens zwei Sequenzen umfasst, die zueinander komplementär sind und wobei ein Sinnstrang eine erste Sequenz und ein Antisinnstrang eine zweite Sequenz umfasst, umfassend einen Komplementaritätsbereich, der zumindest zu einem Teil einer mRNA, die PCSK9 kodiert, vollständig komplementär ist, wobei der Komplementaritätsbereich weniger als 30 Nukleotide lang ist, wobei die dsRNA nach Inkontaktbringen mit einer Zelle, die PCSK9 exprimiert, die Expression des PCSK9-Gens um mindestens 40% inhibiert, und wobei der Teil einer mRNA, die PCSK9 kodiert, aus der Sequenz UCAUAGGCCUGGAGUUUAU besteht.

2. dsRNA nach Anspruch 1, wobei die dsRNA mindestens ein modifiziertes Nukleotid umfasst.

3. dsRNA nach Anspruch 1 oder 2, wobei das modifizierte Nukleotid ausgewählt ist aus der Gruppe eines 2'-O-Methyl-modifizierten Nukleotids, eines Nukleotids umfassend eine 5'-Phosphorothioat-Gruppe, eines endständigen Nukleotids, das mit einem Cholesterylderivat oder einer Dodecansäure-bisdecylamid-Gruppe verbunden ist, eines 2'-Desoxy-2'-fluoro-modifizierten Nukleotids, eines 2'-Desoxy-modifizierten Nukleotids, eines "locked" Nukleotids, eines abasischen Nukleotids, eines 2'-Amino-modifizierten Nukleotids, eines 2'-Alkyl-modifizierten Nukleotids, eines Morpholino-Nukleotids, eines Phosphoramidats und eines Nukleotids, das eine nicht-natürliche Base umfasst.

4. dsRNA nach Anspruch 2 oder 3, wobei die erste Sequenz die Sequenz der SEQ ID NO:453 und die zweite Sequenz die Sequenz der SEQ ID NO:454 ist.

5. Pharmazeutische Zusammensetzung zum Inhibieren der Expression des PCSK9-Gens in einem Organismus, umfassend eine dsRNA und einen pharmazeutisch verträglichen Träger, wobei die dsRNA wie in einem der Ansprüche 1 bis 4 definiert ist.

6. *In-vitro*-Verfahren zum Inhibieren der Expression des PCSK9-Gens in einer Zelle, wobei das Verfahren umfasst:

(a) Einbringen einer doppelsträngigen Ribonukleinsäure (dsRNA) in die Zelle, wobei die dsRNA wie in einem der Ansprüche 1 bis 4 definiert ist; und
(b) Aufrechterhalten der in Schritt (a) produzierten Zelle für eine Zeit, die ausreicht, einen Abbau des mRNA-Transkripts des PCSK9-Gens zu erhalten, wodurch die Expression des PCSK9-Gens in der Zelle inhibiert wird.

7. dsRNA zum Behandeln, Vorbeugen oder Handhaben pathologischer Prozesse, das durch Herabregulieren der PCSK9-Genexpression vermittelt werden kann, wobei die dsRNA wie in einem der Ansprüche 1 bis 4 definiert ist.

8. Vektor zum Inhibieren der Expression des PCSK9-Gens in einer Zelle, wobei der Vektor eine regulatorische Sequenz umfasst, die mit einer Nukleotidsequenz funktionell verbunden ist, die mindestens einen Strang einer dsRNA kodiert, wobei die dsRNA wie in einem der Ansprüche 1 bis 4 definiert ist.

9. Isolierte Zelle, umfassend die dsRNA nach einem der Ansprüche 1 bis 4 oder den Vektor nach Anspruch 8.

10. Doppelsträngige Ribonukleinsäure (dsRNA) zum Reduzieren des Expressionsspiegels eines menschlichen PCSK9-Gens in einer Zelle, wobei die dsRNA wie in einem der Ansprüche 1 bis 4 definiert ist.

11. dsRNA nach Anspruch 10, wobei das Inkontaktbringen den Expressionsspiegel des PCSK9-Gens reduziert.

12. dsRNA nach Anspruch 10, wobei das Inkontaktbringen *in vitro* bei 30nM oder weniger durchgeführt wird.

13. Pharmazeutische Zusammensetzung zum Reduzieren des Expressionsspiegels des PCSK9-Gens in einem Organismus, umfassend die dsRNA nach Anspruch 10 und einen pharmazeutisch verträglichen Träger.

14. dsRNA nach Anspruch 10 für das Behandeln einer mit PCSK9 in Zusammenhang stehenden Störung.

15. dsRNA nach Anspruch 14, wobei die mit PCSK9 in Zusammenhang stehende Störung eine Hyperlipidämie ist.

Revendications

1. Acide ribonucléique double brin (ARNdb) pour l'inhibition de l'expression d'un gène PCSK9 humain dans une cellule, dans lequel ledit ARNdb comprend au moins deux séquences qui sont complémentaires l'une de l'autre et dans lequel un brin sens comprend une première séquence et un brin antisens comprend une seconde séquence comprenant une région de complémentarité qui est entièrement complémentaire avec au moins une partie d'un ARNm codant pour PCSK9, dans lequel ladite région de complémentarité fait moins de 30 nucléotides en longueur et dans lequel ledit ARNdb, lorsqu'il est mis en contact avec une cellule exprimant ladite PCSK9, inhibe l'expression dudit gène PCSK9 d'au moins 40%, et dans lequel ladite partie d'un ARNm codant pour PCSK9 consiste en la séquence UCAUAGGCCUGGAGUUUAU.

2. ARNdb selon la revendication 1, dans lequel ledit ARNdb comprend au moins un nucléotide modifié.

3. ARNdb selon la revendication 1 ou 2, dans lequel ledit nucléotide modifié est choisi au sein du groupe constitué d'un nucléotide 2'-O-méthyle modifié, un nucléotide comprenant un groupe 5'-phosphorothioate, un nucléotide terminal lié à un dérivé de cholestéryle ou un groupe bisdécylamide d'acide dodécanoïque, un nucléotide 2'-désoxy-2'-fluoro modifié, un nucléotide 2'-désoxy modifié, un nucléotide verrouillé, un nucléotide abasique, un nucléotide 2'-amino modifié, un nucléotide 2'-alkyle modifié, un nucléotide morpholino, un phosphoramidate, et une base non naturelle comprenant un nucléotide.

4. ARNdb selon la revendication 2 ou 3, dans lequel ladite première séquence est la séquence de SEQ ID NO: 453 et ladite seconde séquence est la séquence de SEQ ID NO : 454.

5. Composition pharmaceutique pour l'inhibition de l'expression du gène PCSK9 dans un organisme, comprenant un ARNdb et un support acceptable sur le plan pharmaceutique, dans laquelle l'ARNdb est tel que défini dans l'une quelconque des revendications 1 à 4.

6. Méthode *in vitro* pour l'inhibition de l'expression du gène PCSK9 dans une cellule, la méthode comprenant :

(a) l'introduction dans la cellule d'un acide ribonucléique double brin (ARNdb), dans laquelle l'ARNdb est tel que défini dans l'une quelconque des revendications 1 à 4 ; et
(b) le maintien de la cellule produite dans l'étape (a) pendant un temps suffisant pour obtenir une dégradation du transcrit ARNm du gène PCSK9, inhibant ainsi l'expression du gène PCSK9 dans la cellule.

7. ARNdb pour le traitement, la prévention ou la gestion de procédés pathologiques qui peuvent être médiés par la régulation à la baisse de l'expression du gène PCSK9, dans lequel l'ARNdb est tel que défini dans l'une quelconque des revendications 1 à 4.

8. Vecteur pour l'inhibition de l'expression du gène PCSK9 dans une cellule, ledit vecteur comprenant une séquence régulatrice liée de façon opérationnelle à une séquence nucléotidique qui code pour au moins un brin d'un ARNdb, dans lequel ledit ARNdb est tel que défini dans l'une quelconque des revendications 1 à 4.

9. Cellule isolée comprenant l'ARNdb selon l'une quelconque des revendications 1 à 4 ou le vecteur selon la revendication 8.

10. Acide ribonucléique double brin (ARNdb) pour la réduction du niveau d'expression d'un gène PCSK9 humain dans une cellule, dans lequel ledit ARNdb est tel que défini dans l'une quelconque des revendications 1 à 4.

11. ARNdb selon la revendication 10, dans lequel ledit contact réduit le niveau d'expression dudit gène PCSK9.

12. ARNdb selon la revendication 10, dans lequel ledit contact est réalisé *in vitro* à 30 nM ou moins.

13. Composition pharmaceutique pour la réduction du niveau d'expression du gène PCSK9 dans un organisme, comprenant l'ARNdb selon la revendication 10 et un support acceptable sur le plan pharmaceutique.

14. ARNdb selon la revendication 10 pour le traitement d'un trouble associé à PCSK9.

15. ARNdb selon la revendication 14, dans lequel ledit trouble associé à PCSK9 est l'hyperlipidémie.

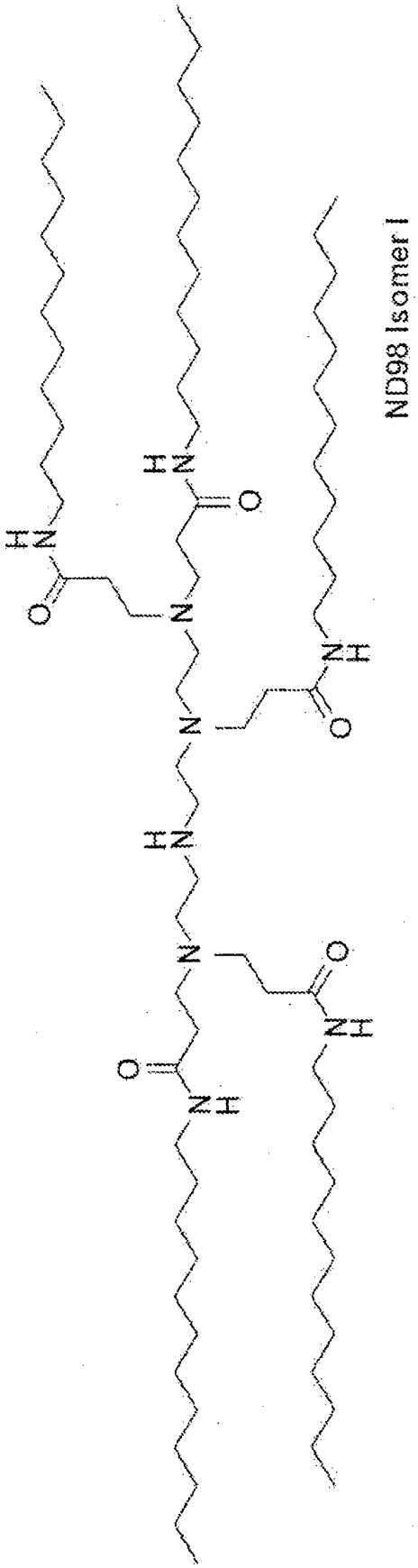

FIG. 1

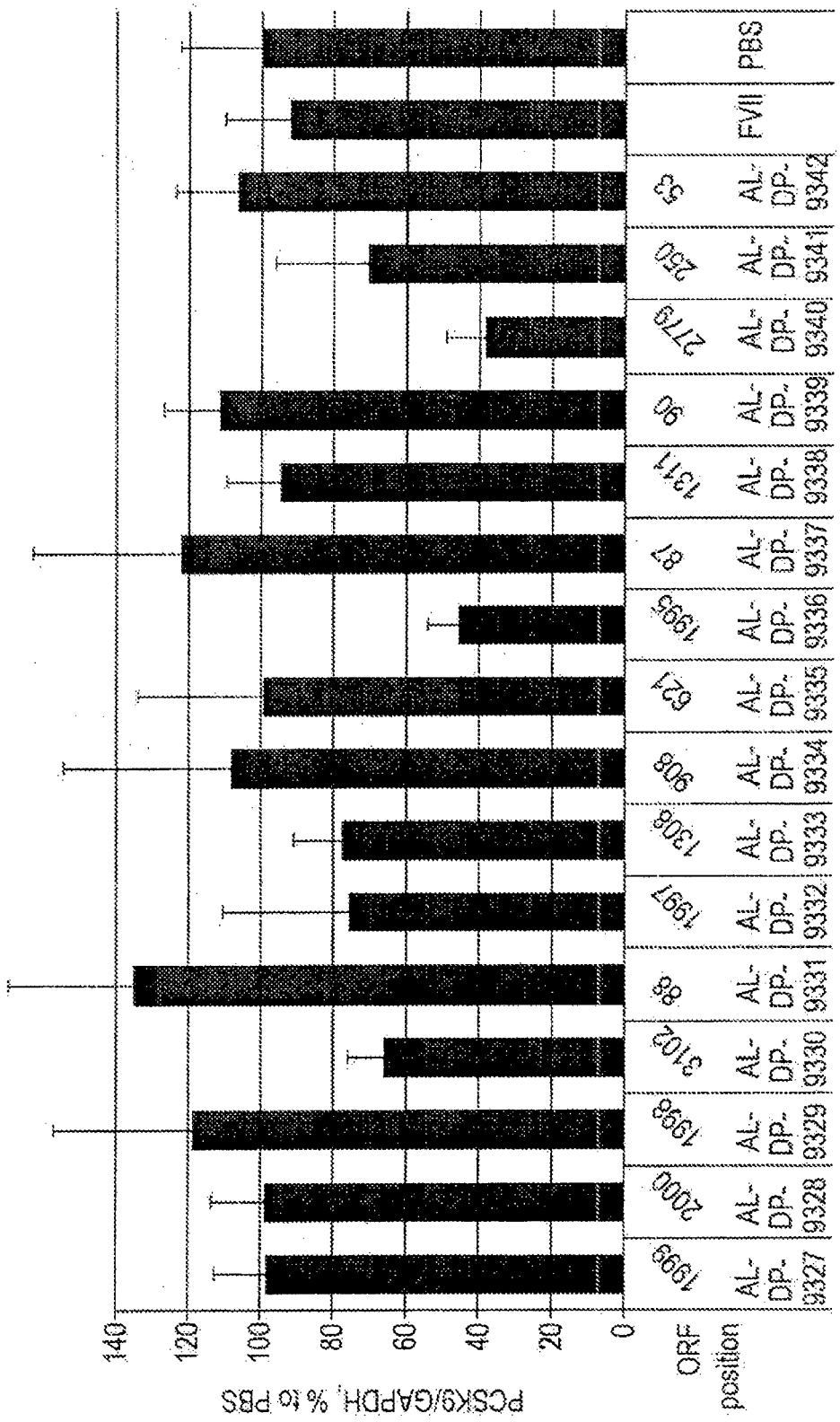
PCSK9/GAPDH, % to PBS
140
120
100
80
60
40
20
0
ORF position
1999
2000
1998
3102
88
1997
1308
908
621
1995
87
1311
90
2779
260
53
PBS
FVII
AL-DP-9327
AL-DP-9328
AL-DP-9329
AL-DP-9330
AL-DP-9331
AL-DP-9332
AL-DP-9333
AL-DP-9334
AL-DP-9335
AL-DP-9336
AL-DP-9337
AL-DP-9338
AL-DP-9339
AL-DP-9340
AL-DP-9341
AL-DP-9342

FIG. 2

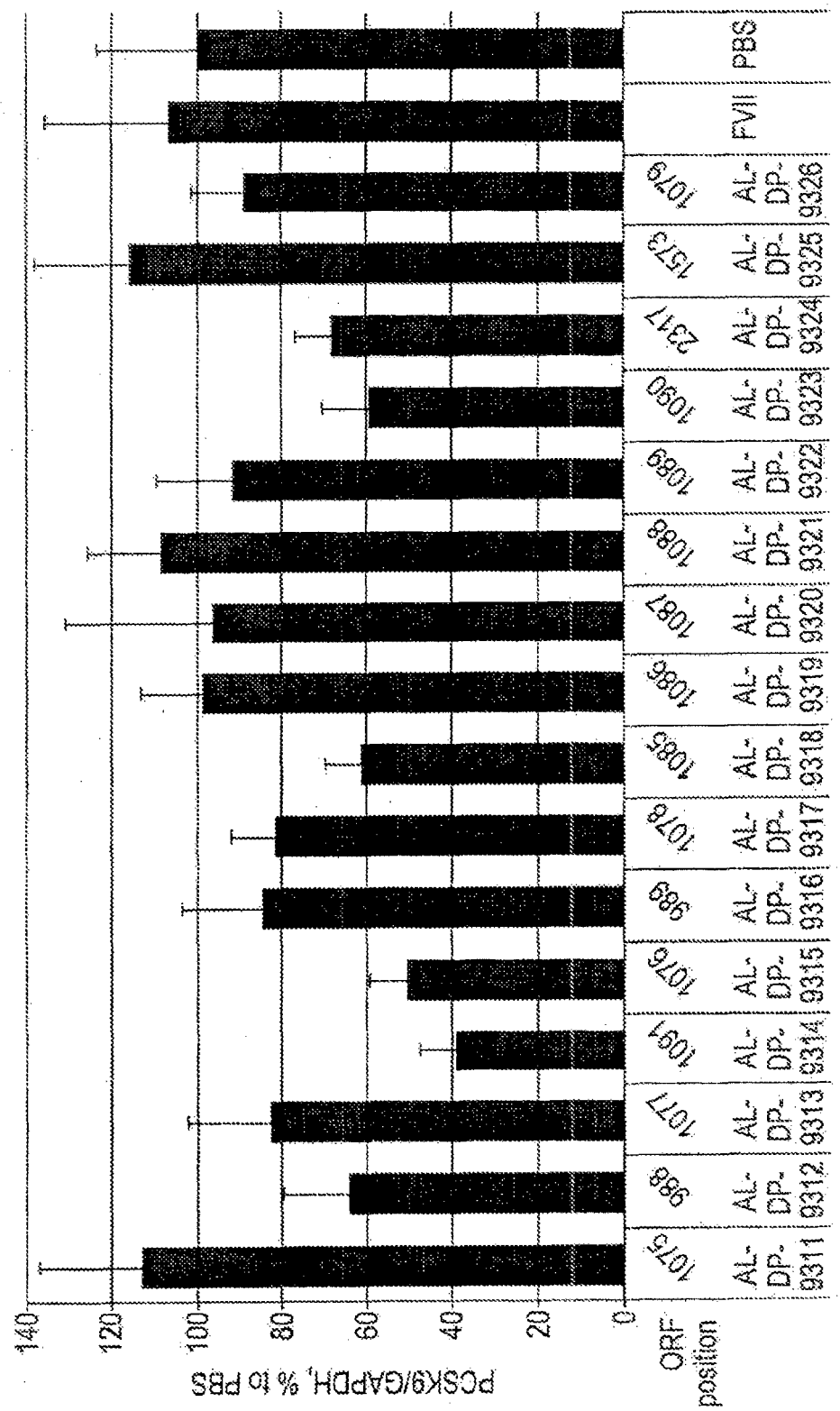
PCSK9/GAPDH, % to PBS
0
20
40
60
80
100
120
140
ORF position
AL-DP-9311
1075
AL-DP-9312
988
AL-DP-9313
1077
AL-DP-9314
1091
AL-DP-9315
1076
AL-DP-9316
989
AL-DP-9317
1078
AL-DP-9318
1085
AL-DP-9319
1086
AL-DP-9320
1087
AL-DP-9321
1088
AL-DP-9322
1089
AL-DP-9323
1090
AL-DP-9324
2317
AL-DP-9325
1573
AL-DP-9326
1079
FVIII
PBS

FIG. 3

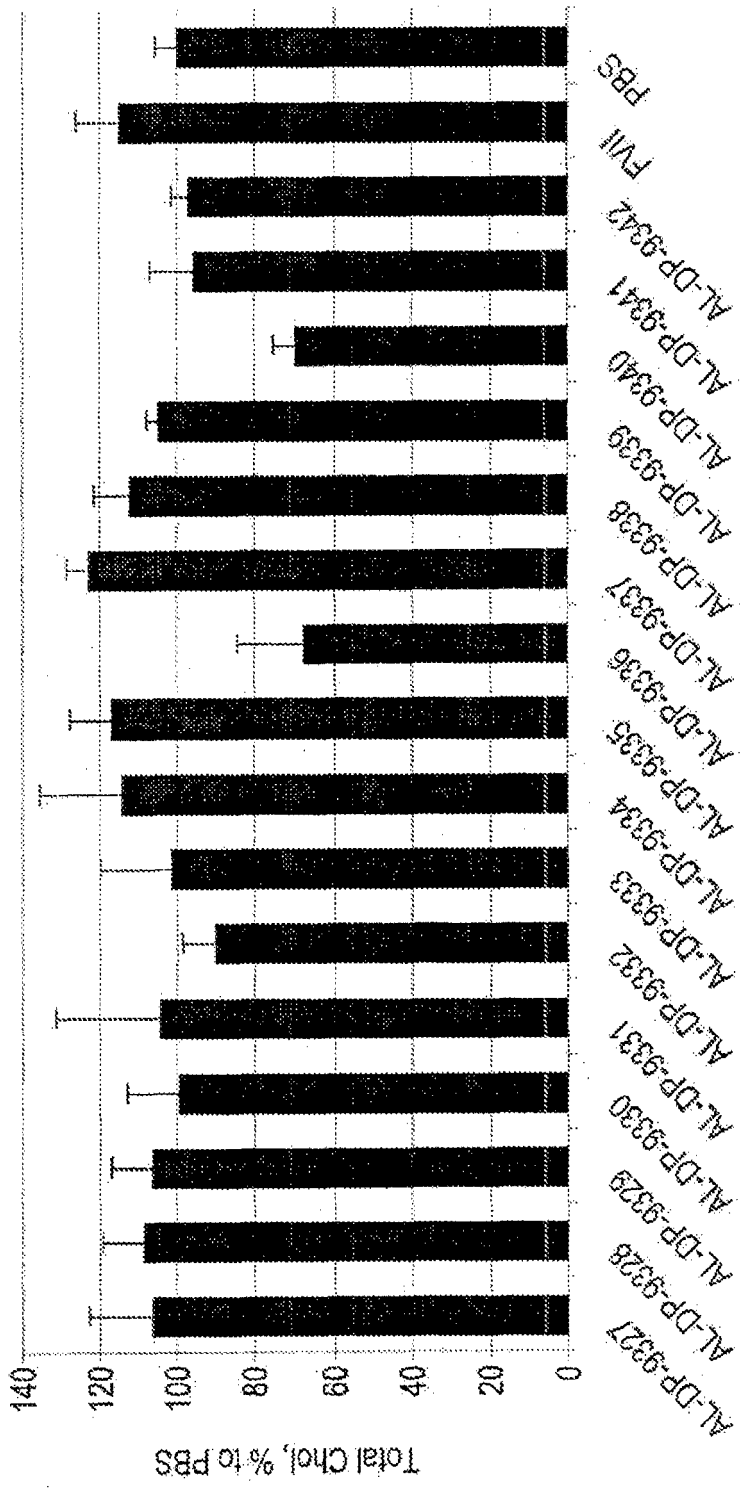
Total Chol, % to PBS
140
120
100
80
60
40
20
0
AL-DP-9327
AL-DP-9328
AL-DP-9329
AL-DP-9330
AL-DP-9331
AL-DP-9332
AL-DP-9333
AL-DP-9334
AL-DP-9335
AL-DP-9336
AL-DP-9337
AL-DP-9338
AL-DP-9339
AL-DP-9340
AL-DP-9341
AL-DP-9342
FVII
PBS

FIG. 4

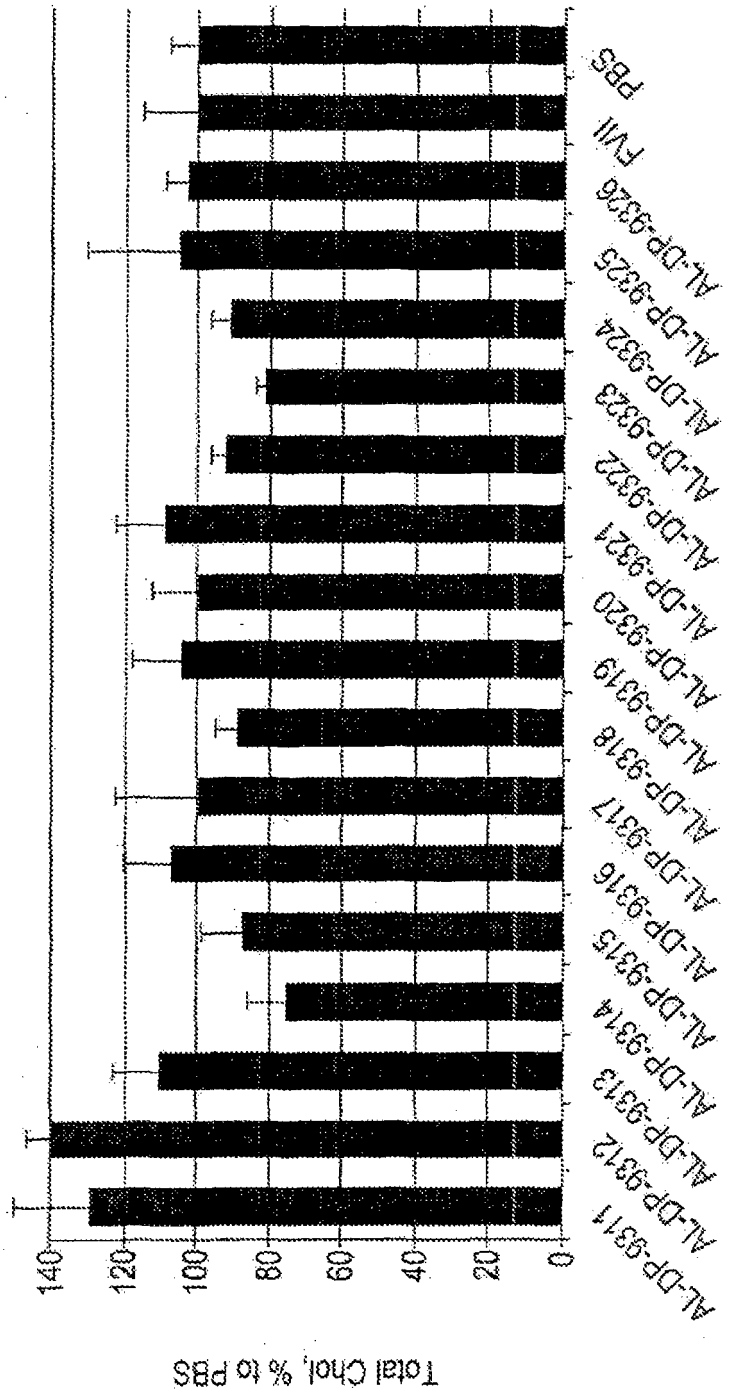
Total Chol, % to PBS
140
120
100
80
60
40
20
0
AL-DP-9311
AL-DP-9312
AL-DP-9313
AL-DP-9314
AL-DP-9315
AL-DP-9316
AL-DP-9317
AL-DP-9318
AL-DP-9319
AL-DP-9320
AL-DP-9321
AL-DP-9322
AL-DP-9323
AL-DP-9324
AL-DP-9325
AL-DP-9326
FVII
PBS

FIG. 5

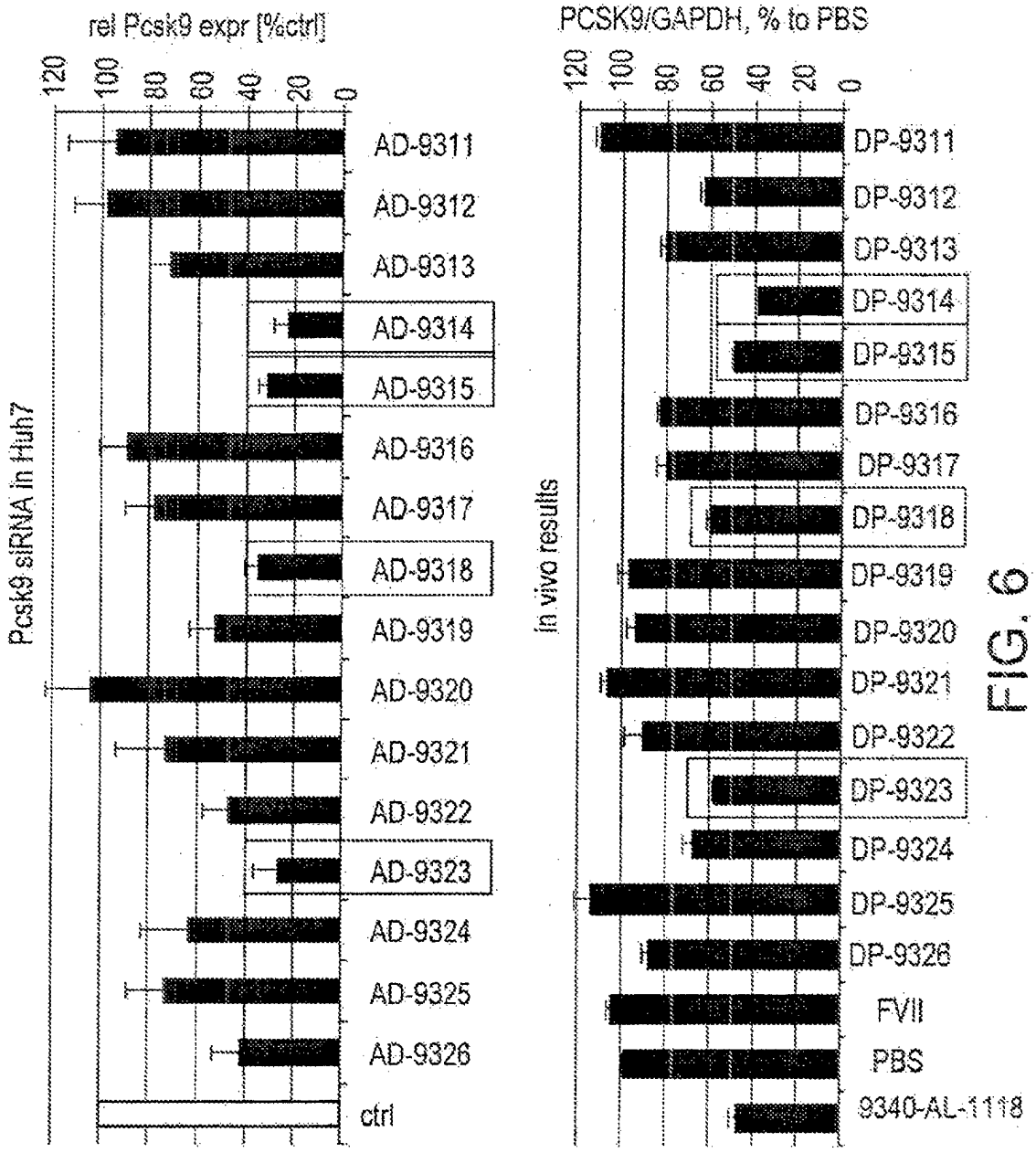
rel Pcsk9 expr [%ctrl]
120
100
80
60
40
20
0
Pcsk9 siRNA in Huh7
AD-9311
AD-9312
AD-9313
AD-9314
AD-9315
AD-9316
AD-9317
AD-9318
AD-9319
AD-9320
AD-9321
AD-9322
AD-9323
AD-9324
AD-9325
AD-9326
ctrl
PCSK9/GAPDH, % to PBS
120
100
80
60
40
20
0
in vivo results
DP-9311
DP-9312
DP-9313
DP-9314
DP-9315
DP-9316
DP-9317
DP-9318
DP-9319
DP-9320
DP-9321
DP-9322
DP-9323
DP-9324
DP-9325
DP-9326
FVII
PBS
9340-AL-1118

FIG. 6

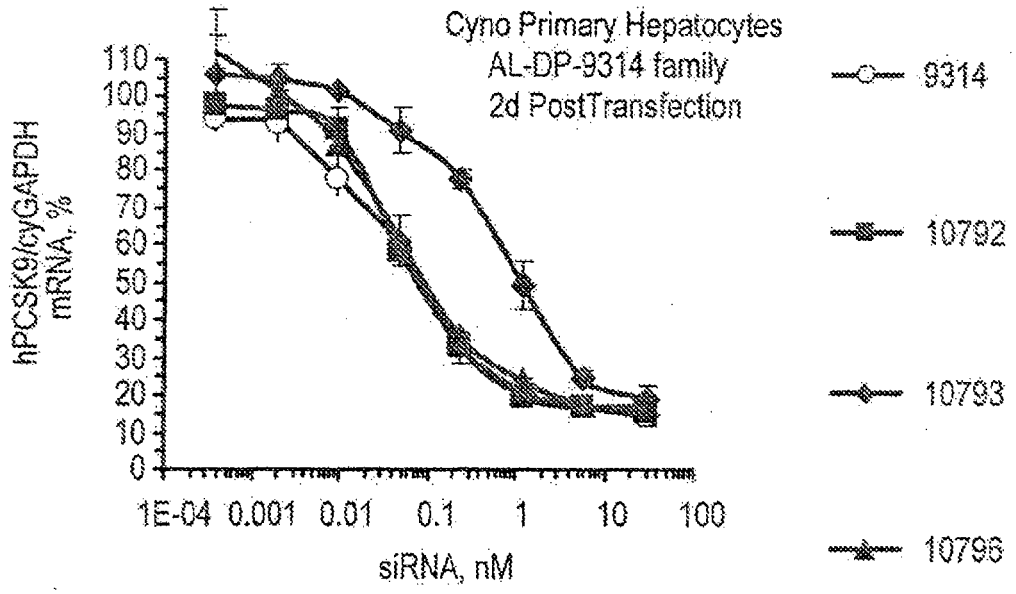
Cyno Primary Hepatocytes
AL-DP-9314 family
2d PostTransfection
hPCSK9/cyGAPDH mRNA, %
110
100
90
80
70
60
50
40
30
20
10
0
1E-04
0.001
0.01
0.1
1
10
100
siRNA, nM
9314
10792
10793
10796

FIG. 7A

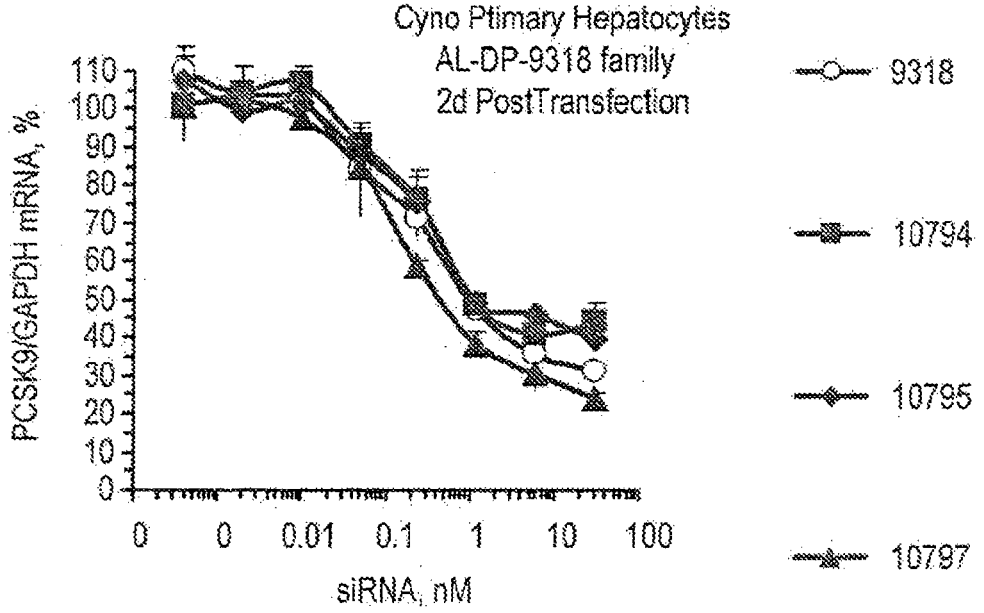
Cyno Ptimary Hepatocytes
AL-DP-9318 family
2d PostTransfection
PCSK9/GAPDH mRNA, %
110
100
90
80
70
60
50
40
30
20
10
0
0
0
0.01
0.1
1
10
100
siRNA, nM
9318
10794
10795
10797

FIG. 7B

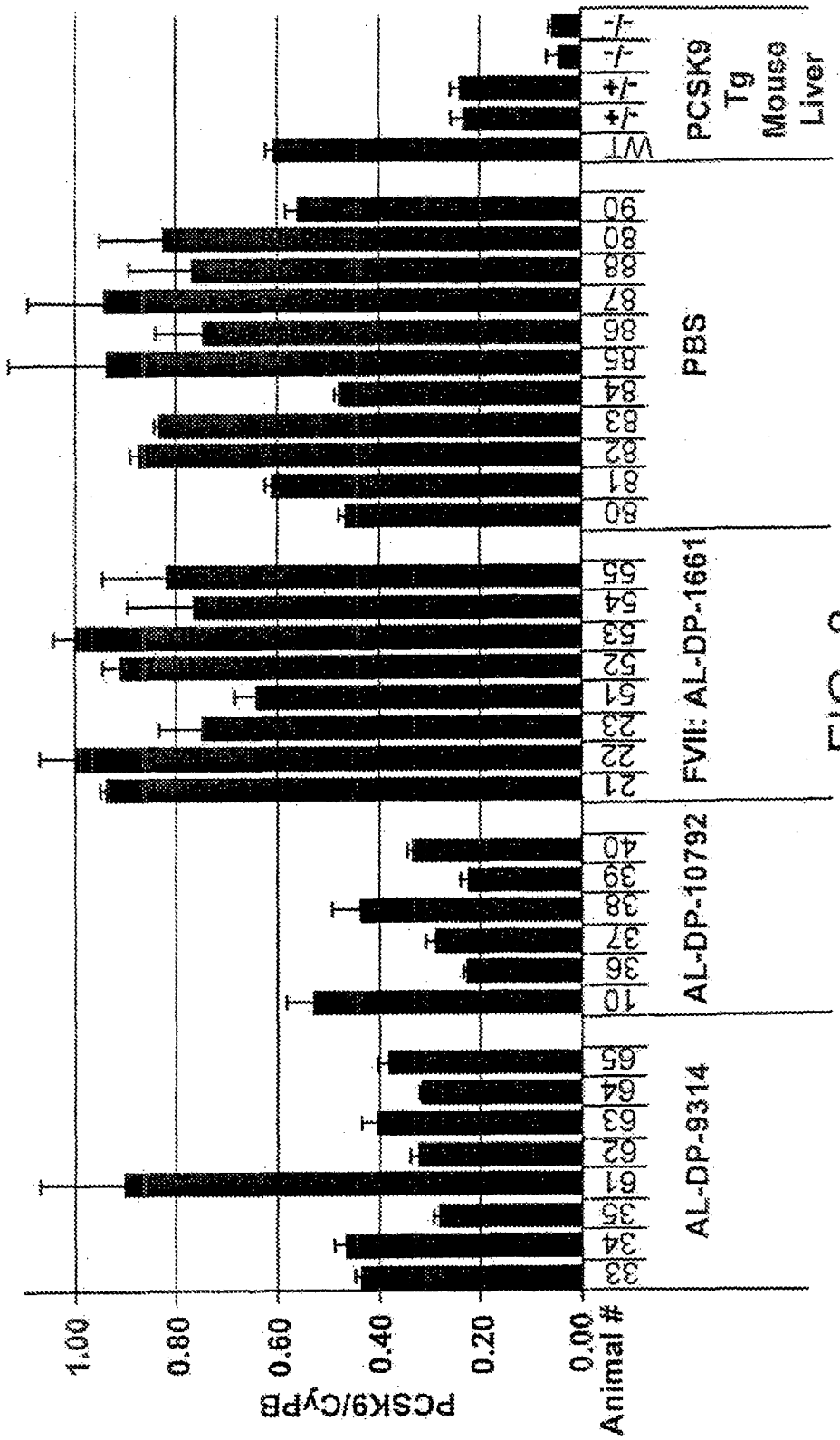
PCSK9/CyPB
1.00
0.80
0.60
0.40
0.20
0.00
Animal #
33
34
35
61
62
63
64
65
AL-DP-9314
10
36
37
38
39
40
AL-DP-10792
21
22
23
51
52
53
54
55
FVII: AL-DP-1661
80
81
82
83
84
85
86
87
88
90
PBS
WT
+/+
+/-
-/-
-/-
PCSK9 Tg Mouse Liver

FIG. 8

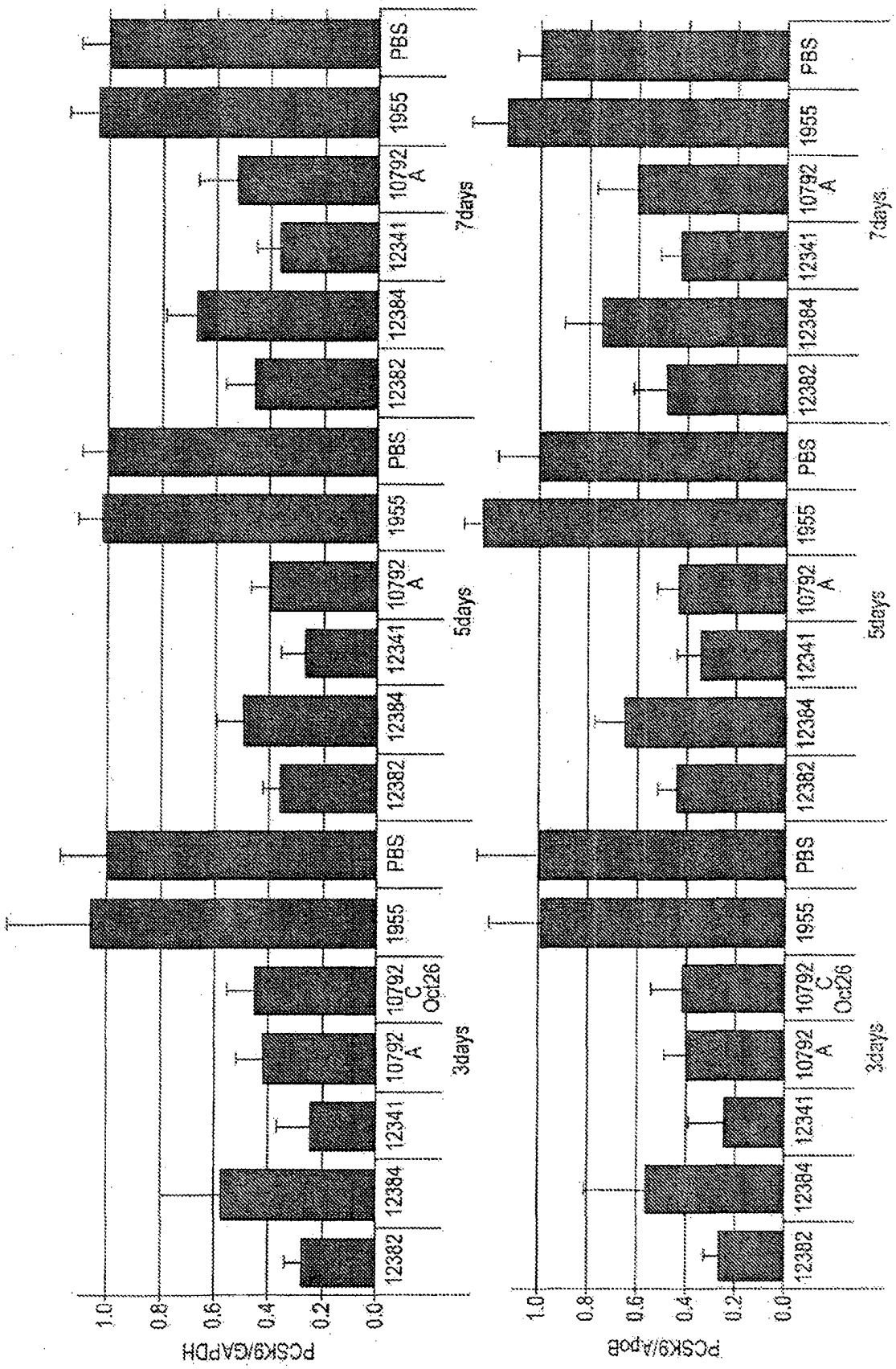
PCSK9/GAPDH
PCSK9/Apob
1.0
0.8
0.6
0.4
0.2
0.0
12382
12384
12341
10792 A
10792 C Oct26
1955
PBS
3days
5days
7days

FIG. 9

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